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(54) Title: VACCINATION AND METHODS AGAINST DISEASES RESULTING FROM PATHOGENIC RESPONSES BY SPECIFIC T CELL POPULATIONS		
(57) Abstract <p>The present invention provides vaccines and a means of vaccinating a vertebrate so as to prevent or control specific T cell mediated pathologies, including autoimmune diseases and the unregulated replication of T cells. The vaccine is composed of a T cell receptor (TCR) or a fragment thereof corresponding to a TCR present on the surface of T cells mediating the pathology. The vaccine fragment can be a peptide corresponding to sequences of TCRs characteristic of the T cells mediating said pathology. Such a peptide can bind to conventional antigens complexed to MHC antigen presenting cells or to superantigens. Means of determining appropriate amino acid sequences for such vaccines are also provided. The vaccine is administered to the vertebrate in a manner that induces an immune response directed against the TCR of T cells mediating the pathology. This immune response down regulates or deletes the pathogenic T cells, thus ablating the disease pathogenesis. The invention additionally provides specific β-chain variable regions of T cell receptors, designated Vβ3, Vβ4, Vβ12, Vβ14 and Vβ17, which are associated with the pathogenesis of autoimmune diseases, such as rheumatoid arthritis (RA) and multiple sclerosis (MS). Also provided are means to detect, prevent and treat RA and MS. Methods of administering DNA or RNA encoding the polypeptides useful as vaccines of the present invention into the tissue cells of an individual is also provided.</p>		

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VACCINATION AND METHODS AGAINST DISEASES RESULTING
FROM PATHOGENIC RESPONSES
BY SPECIFIC T CELL POPULATIONS

BACKGROUND OF THE INVENTION

5 This invention relates to the immune system and, more specifically, to methods of modifying pathological immune responses.

 Higher organisms are characterized by an immune system which protects them against invasion by
10 potentially deleterious substances or microorganisms. When a substance, termed an antigen, enters the body, and is recognized as foreign, the immune system mounts both an antibody-mediated response and a cell-mediated
15 response. Cells of the immune system termed B lymphocytes, or B cells, produce antibodies that specifically recognize and bind to the foreign substance. Other lymphocytes termed T lymphocytes, or T cells, both effect and regulate the cell-mediated response resulting eventually in the elimination of the antigen.

20 A variety of T cells are involved in the cell-mediated response. Some induce particular B cell clones to proliferate and produce antibodies specific for the antigen. Others recognize and destroy cells presenting foreign antigens on their surfaces. Certain T cells

regulate the response by either stimulating or suppressing other cells.

While the normal immune system is closely regulated, aberrations in immune response are not uncommon. In some instances, the immune system functions inappropriately and reacts to a component of the host as if it were, in fact, foreign. Such a response results in an autoimmune disease, in which the host's immune system attacks the host's own tissue. T cells, as the primary regulators of the immune system, directly or indirectly effect such autoimmune pathologies.

Numerous diseases are believed to result from autoimmune mechanisms. Prominent among these are rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, Type I diabetes, myasthenia gravis and pemphigus vulgaris. Autoimmune diseases affect millions of individuals world-wide and the cost of these diseases, in terms of actual treatment expenditures and lost productivity, is measured in billions of dollars annually. At present, there are no known effective treatments for such autoimmune pathologies. Usually, only the symptoms can be treated, while the disease continues to progress, often resulting in severe debilitation or death.

In other instances, lymphocytes replicate inappropriately and without control. Such replication results in a cancerous condition known as a lymphoma. Where the unregulated lymphocytes are of the T cell type, the tumors are termed T cell lymphomas. As with other malignancies, T cell lymphomas are difficult to treat effectively.

Thus, a long-felt need exists for an effective means of curing or ameliorating T cell mediated

pathologies. Such a treatment should ideally control the inappropriate T cell response, rather than merely reducing the symptoms. The present invention satisfies this need and provides related advantages as well.

5

Summary of the Invention

The present invention provides vaccines and a means of vaccinating a vertebrate so as to prevent or control specific T cell mediated pathologies. The vaccine is composed of a substantially pure T cell
10 receptor (TCR) or an immunogenic fragment thereof corresponding to a TCR present on the surface of T cells mediating the pathology. The vaccine fragment can be a peptide corresponding to sequences of TCRs characteristic of the T cells mediating said pathology.

15 The invention additionally provides specific β -chain variable regions and their immunogenic segments, and in particular three T cell receptors, designated VB3, VB14 and VB17, which are associated with the pathogenesis of autoimmune diseases, for example rheumatoid arthritis
20 (RA) and multiple sclerosis (MS). Additional VDJ junctional (CDR3) regions associated with other autoimmune diseases are also provided. The present invention further relates to means for detecting, preventing and treating RA, MS and other autoimmune
25 diseases.

The invention further provides methods of preventing or treating T cell mediated pathologies, including RA and MS, by gene therapy. In these methods, pure DNA or RNA encoding for a TCR, an immunogenic
30 fragment thereof or an anti-idiotypic antibody having an internal image of a TCR or an immunogenic fragment is administered to an individual. V ctors containing the

DNA or RNA and compositions containing such vectors are also provided for use in these methods.

Brief Description of the Figures

Figure 1 shows the variable region sequences of VB3, VB14 and VB17. The boxed segments depict the CDR1, CDR2 and CDR4 hypervariable regions of each VB chain. The sequences between the CDR2 and CDR4 regions represent an overlap between these two hypervariable regions.

Figure 2(A) shows the location of primers used in polymerase chain reaction amplification of T cell receptor β -chain genes, and 2(B) shows primer sequences used in polymerase chain reaction.

Figure 3 shows the location and sequence of primers used in polymerase chain reaction amplification of HLA-DR B₁ genes. Also shown are HLA-DR allele specific oligonucleotides.

Detailed Description of the Invention

The invention generally relates to vaccines and their use for preventing, ameliorating or treating T cell-mediated pathologies, such as autoimmune diseases and T cell lymphomas. Vaccination provides a specific and sustained treatment which avoids problems associated with other potential avenues of therapy.

As used herein, the term "T cell-mediated pathology" refers to any condition in which an inappropriate T cell response is a component of the pathology. The term is intended to encompass both T cell mediated autoimmune diseases and diseases resulting from unregulated clonal T cell replication. In addition, the term is intended to include both diseases directly

mediated by T cells and those, such as myasthenia gravis, which are characterized primarily by damage resulting from antibody binding, and also diseases in which an inappropriate T cell response contributes to the production of those antibodies.

As used herein, "substantially the amino acid sequence," or "substantially the sequence" when referring to an amino acid sequence, means the described sequence or other sequences having any additions, deletions or substitutions that do not substantially effect the ability of the sequence to elicit an immune response against the desired T cell receptor sequence. Such sequences commonly have many other sequences adjacent to the described sequence. A portion or segment of the described immunizing sequence can be used so long as it is sufficiently characteristic of the desired T cell receptor or fragment thereof to cause an effective immune response against desired T cell receptors, but not against undesired T cell receptors. Such variations in the sequence can easily be made, for example by synthesizing an alternative sequence. The alternate sequence can then be tested, for example by immunizing a vertebrate, to determine its effectiveness.

As used herein, the term "fragment" means an immunogenically effective subset of the amino acid sequence that comprises a TCR. The term is intended to include such fragments in conjunction with or combined with additional sequences or moieties, as for example where the peptide is coupled to other amino acid sequences or to a carrier. The terms "fragment" and "peptide" can, therefore, be used interchangeably since a peptide will be the most common fragment of the T cell receptor. Each fragment of the invention can have an altered sequence, as described above for the term "substantially the sequence."

Reference herein to a "fragment," "portion" or "segment" of a T cell receptor does not mean that the composition must be derived from intact T cell receptors. Such "fragments," "portions" or "segments" can be produced
5 by various means well-known to those skilled in the art, such as, for example, manual or automatic peptide synthesis, various methods of cloning or enzymatic treatment of a whole TCR.

As used herein when referring to the
10 relationship between peptide fragments of the invention and sequences of TCRs, "corresponding to" means that the peptide fragment has an amino acid sequence which is sufficiently homologous to a TCR sequence or fragment thereof to stimulate an effective regulatory response in
15 the individual. The sequence, however, need not be identical to the TCR sequence as shown, for instance, in Examples II and III.

By "immunogenically effective" is meant an amount of the T cell receptor or fragment thereof which
20 is effectively elicits an immune response to prevent or treat a T cell mediated pathology or an unregulated T cell clonal replication in an individual. Such amounts will vary between species and individuals depending on many factors for which one skilled in the art can
25 determine.

As used herein, "binding partner" means a compound which is reactive with a TCR. Generally, this compound will be a Major Histocompatibility Antigen (MHC) but can be any compound capable of directly or indirectly
30 stimulating T cell activation or proliferation when bound to the TCR. Such compounds can also, for example, be a superantigen that binds to a superantigen binding site on the TCR.

As used herein, "individual" means any vertebrate, including humans, capable of having a T cell mediated pathology or unregulated clonal T cell replication and is used interchangeably with
5 "vertebrate."

As used herein, "ligand" means any molecule that reacts with another molecule to form a complex.

As used herein, "selectively binds" means that a molecule binds to one type of molecule or related group
10 of molecules, but not substantially to other types of molecules. In relation to VBs, "selective binding" indicates binding to TCRs or fragments thereof containing a specific VB without substantial cross-reactivity with other TCRs that lack the specific VB.

15 The immune system is the primary biological defense of the host (self) against potentially pernicious agents (non-self). These pernicious agents may be pathogens, such as bacteria or viruses, as well as modified self cells, including virus-infected cells,
20 tumor cells or other abnormal cells of the host. Collectively, these targets of the immune system are referred to as antigens. The recognition of antigen by the immune system rapidly mobilizes immune mechanisms to destroy that antigen, thus preserving the sanctity of the
25 host environment.

The principal manifestations of an antigen-specific immune response are humoral immunity (antibody mediated) and cellular immunity (cell mediated). Each of these immunological mechanisms are initiated through the
30 activation of helper (CD4+) T Cells. These CD4+ T cells in turn stimulate B cells, primed for antibody synthesis by antigen binding, to proliferate and secrete antibody. This secreted antibody binds to the antigen and

facilitates its destruction by other immune mechanisms. Similarly, CD4+ T cells provide stimulatory signals to cytotoxic (CD8+) T cells that recognize and destroy cellular targets (for example, virus infected cells of the host). Thus, the activation of CD4+ T cells is the proximal event in the stimulation of an immune response. Therefore, elaboration of the mechanisms underlying antigen specific activation of CD4+ T cells is crucial in any attempt to selectively modify immunological function.

T cells owe their antigen specificity to the T cell receptor (TCR) which is expressed on the cell surface. The TCR is a heterodimeric glycoprotein, composed of two polypeptide chains, each with a molecular weight of approximately 45 kD. Two forms of the TCR have been identified. One is composed of an alpha chain and a beta chain, while the second consists of a gamma chain and a delta chain. Each of these four TCR polypeptide chains is encoded by a distinct genetic locus containing multiple discontinuous gene segments. These include variable (V) region gene segments, joining (J) region gene segments and constant (C) region gene segments. Beta and delta chains contain an additional element termed the diversity (D) gene segment. Since D segments and elements are found in only some of the TCR genetic loci, and polypeptides, further references herein to D segments and elements will be in parentheses to indicate the inclusion of these regions only in the appropriate TCR chains. Thus, V(D)J refers either to VDJ sequences of chains which have a D region or refers to VJ sequences of chains lacking D regions.

With respect to the beta chain of the variable region referred to as a V β , the nomenclature used herein to identify specific V β s follows that of Kimura et al., Eur. J. Immuno. 17:375-383 (1987), with the exception

that the VB14 h rein corresponds to VB3.3 of Kimura et al.

During lymphocyte maturation, single V, (D) and J gene segments are rearranged to form a functional gene that determines the amino acid sequence of the TCR expressed by that cell. Since the pool of V, (D) and J genes which may be rearranged is multi-membered and since individual members of these pools may be rearranged in virtually any combination, the complete TCR repertoire is highly diverse and capable of specifically recognizing and binding the vast array of binding partners to which an organism may be exposed. However, a particular T cell will have only one TCR molecule and that TCR molecule, to a large degree if not singly, determines the specificity of that T cell for its binding partner.

Animal models have contributed significantly to the understanding of the immunological mechanisms of autoimmune disease. One such animal model, experimental allergic encephalomyelitis (EAE), is an autoimmune disease of the central nervous system that can be induced in mice and rats by immunization with myelin basic protein (MBP). The disease is characterized clinically by paralysis and mild wasting and histologically by a perivascular mononuclear cell infiltration of the central nervous system parenchyma. The disease pathogenesis is mediated by T cells having specificity for MBP. Multiple clones of MBP-specific T cells have been isolated from animals suffering from EAE and have been propagated in continuous culture. After in vitro stimulation with MBP, these T cell clones rapidly induce EAE when adoptively transferred to healthy hosts. Importantly, these EAE-inducing T cells are specific not only for the same antigen (MBP), but usually also for a single epitope on that antigen. These observations indicate that discrete

populations of autoaggressive T cells are responsible for the pathogenesis of EAE.

Analysis of the TCRs of EAE-inducing T cells has revealed restricted heterogeneity in the structure of these disease-associated receptors. In one analysis of 33 MBP-reactive T cells, only two alpha chain V region gene segments and a single alpha chain J region gene segment were found. Similar restriction of beta chain TCR gene usage was also observed in this T cell population. Only two beta chain V region segments and two J region gene segments were found. More importantly, approximately eighty percent of the T cell clones had identical amino acid sequences across the region of beta chain V-D-J joining. These findings confirm the notion of common TCR structure among T cells with similar antigen specificities and indicate that the TCR is an effective target for immunotherapeutic strategies aimed at eliminating the pathogenesis of EAE.

Various attempts have been made to exploit the antigen specificity of autoaggressive T cells in devising treatment strategies for EAE. For example, passive administration of monoclonal antibodies specific for TCRs present on EAE-inducing T cells has been employed. In the mouse model of EAE, infusion of a monoclonal antibody specific for VB8, the major beta chain V region gene of by MBP-specific T cells, reduced the susceptibility of mice to subsequent EAE induction as described in Acha-Orbea et al., Cell 54:263-273 (1988) and Urban et al., Cell 54:577-592 (1988). Similar protection has been demonstrated in rat EAE with monoclonal antibody reactive with an unidentified idiotypic determinant of the TCR on MBP specific T cells (Burns et al., J. Exp. Med. 169:27-39 (1989)). While passive antibody therapy appears to have some ameliorative effect on EAE susceptibility, it is fraught with potential problems. The protection

afforded is transient, thus requiring repeated administration of the antibody. Multiple infusions of antibody increase the chances the host will mount an immune response to the administered antibody,
5 particularly if it is raised in a xenogeneic animal. Further, an antibody response to a pathogenic T cell clone represents only one element in the complete immune response and neglects the potential contributions of cellular immunity in resolving the autoreactivity.

10 The role of cellular immunity in reducing the activity of autoaggressive T cells in EAE has been examined and potential therapies suggested. In a manner similar to the passive antibody approach, regulatory T cells have been derived ex vivo and readministered for
15 immunotherapy. For example, a CD8+ T cell line was recently isolated from convalescing rats in which EAE had been induced by adoptive transfer of an MBP-specific CD4+ T cell line. Sun et al., Nature, 332:843-845 (1988). This CD8+ T cell line displayed cytolytic activity in
20 vitro for the CD4+ T cell used to induce disease. Moreover, adoptive transfer of this CTL line reduced the susceptibility of recipient rats to subsequent challenge with MBP. Lider et al., Science, 239:181-183 (1988) also report the isolation of CD8+ T cells with suppressive
25 activity for EAE-inducing T cells. These CD8+ cells were isolated from rats vaccinated with attenuated disease-inducing T cell clones and, though they showed no cytolytic activity in vitro, they suppressed MBP-driven proliferation of EAE-inducing T cells. Although these
30 studies indicate that the CD8+ T cells could downregulate EAE, it is hard to reconcile a major role for these selected CD8+ CTLs in the long-term resistance of the recovered rats since Sedgwick, et al., (Eur. J. Immunol., 18:495-502 (1988)) have clearly shown that depletion of
35 CD8+ cells with monoclonal antibodies does not affect the disease process or recovery.

In the experiments of Sun et al., and Lider et al., described above, the administration of ex vivo derived regulatory T cells overcomes the major obstacle of passive antibody therapy in that it permits a
5 regulatory response in vivo of prolonged duration. However, it requires in vitro cultivation with attenuated disease-inducing T cells to develop clones of such regulatory T cells, a costly and labor intensive process. Further, in an outbred population such as humans, MHC
10 non-identity among individuals makes this a highly individualized therapeutic strategy. Regulatory clones need to be derived for each individual patient and then re-administered only to that patient to avoid potential graft versus host reactions.

15 Direct vaccination with attenuated disease-inducing T cell clones also has been employed as a therapy for EAE. MBP-specific T cells, capable of transferring disease, have been attenuated by gamma irradiation or chemical fixation and used to vaccinate
20 naive rats. In some cases, vaccinated animals exhibited resistance to subsequent attempts at EAE induction (Lider et al., supra; see Cohen and Weiner, Immunol. Today 9:332-335 (1988) for review). The effectiveness of such vaccination, however, is inconsistent and the degree of
25 protection is highly variable. T cells contain a multitude of different antigens which induce an immune response when the whole T cell is administered as a vaccine. This phenomenon has been demonstrated by Offner et al., J. Neuroimmunol., 21:13-22 (1989) by showing that
30 immunization with whole T cells increased the delayed type hypersensitivity (DTH) response, as measured by ear swelling, to those T cells in an incremental manner as the number of vaccinations increased. However, positive DTH responses were found in both protected and non-
35 protected animals. Rats responded similarly to both th vaccinating encephalitog nic T cells and control T cells.

Conversely, vaccination with PPD-specific T cells from a PPD-specific T cell line induced DTH to the vaccinating cells as well as to an encephalitogenic clone even though no protection was observed. The similar response of vaccinated rats to both disease-inducing and control cells, as quantified by delayed-type hypersensitivity (a measure of cell-mediated immunity), indicates that numerous antigens on these T cells are inducing immune responses. Thus, vaccination with attenuated disease-inducing T cells suffers from a lack of specificity for the protective antigen on the surface of that T cell, as well as, variable induction of immunity to that antigen. As a candidate for the treatment of human diseases, vaccination with attenuated T cells is plagued by the same labor intensiveness and need for individualized therapies as noted above for infusion of CD8+ cells.

An alternative mechanism for T cell activation has been suggested in which endogenous and exogenous superantigens have been shown to mediate T-cell stimulation as described, for example, in White et al, Cell 56:27-35 (1989) and Janeway, Cell 63:659-661 (1990).

As used herein, "superantigens" means antigens or fragments thereof that bind preferentially to T cells at specific sites on the β chain of a TCR and stimulate T cells at very high frequency rate. Such superantigens can be endogenous or exogenous. "Frequency" refers to the proportion of T cells responding to antigens and ranges from about 1/5 to 1/100 in response to superantigens. Thus, superantigens are distinguishable from conventional antigens, which have a much lower T cell response frequency rate ranging from about $1/10^4$ to $1/10^6$. Superantigens activate T cells by binding to specific VBs. The superantigen binding sites of various TCRs have been distinguished from the conventional hypervariable regions (CDRs) of TCRs. These CDRs

represent the regions of TCRs thought to be responsible for binding conventional antigens that are complexed to MHC.

The present invention provides an effective
5 method of immunotherapy for T cell mediated pathologies, including autoimmune diseases, which avoids many of the problems associated with previously suggested methods of treatment. By vaccinating, rather than passively
administering heterologous antibodies, the host's own
10 immune system is mobilized to suppress the autoaggressive T cells. Thus, the suppression is persistent and may involve any or all immunological mechanisms in effecting that suppression. This multi-faceted response is more effective than the uni-dimensional suppression achieved
15 by passive administration of monoclonal antibodies or ex vivo-derived regulatory T cell clones which requires a highly individualized therapeutic approach because of MHC non-identity among humans in order to avoid graft versus host reactions. The methods of the present invention are
20 also more effective than vaccination with attenuated disease-inducing T cells that lack specificity for the protective antigen on the surface of a particular T cell as well as the variable induction of immunity to that antigen. In addition, vaccination with attenuated T
25 cells is plagued by the same labor intensiveness and need for individualized therapies as noted above for ex vivo derived regulatory T cell clones.

As they relate to autoimmune disease, the vaccine peptides of the present invention comprise TCRs
30 or immunogenic fragments thereof from specific T cells that mediate autoimmune diseases. The vaccines can be whole TCRs substantially purified from T cell clones, individual T cell receptor chains (for example, alpha, beta, etc.) or portions of such chains, either alone or
35 in combination. The vaccine can be homogenous, for

example, a single peptide, or can be composed of more than one type of peptide, each of which corresponds to a different portion of the TCR. Further, these peptides can be from different TCRs that contribute to the T cell mediated pathology. These vaccine peptides can be of variable length so long as they can elicit a regulatory response. Preferably, such peptides are between about 5 - 100 amino acids in length, and more preferably between about 6 - 25 amino acids in length.

10 In a specific embodiment, the immunizing peptide can have the amino acid sequence of a β -chain VDJ region when the subject has MS or RA. Any immunogenic portion of these peptides can be effective, particularly a portion having substantially the sequence SGDQGGNE (SEQ ID No. 1) or CAIGSNTE (SEQ ID No. 2) of VB4 and VB12, respectively, for MS or substantially the sequence ASSLGGA VSYN (SEQ ID No. 3), ASSLGGEETQYF (SEQ ID No. 4), ASSLGGFETQYF (SEQ ID No. 5) or ASSLGGTEAFF (SEQ ID No. 6) or RA. Thus, amino acid substitutions can be made which do not destroy the immunogenicity of the peptide. Additionally, this peptide can be linked to a carrier to further increase its immunogenicity. Alternatively, whole T cell receptors or TCR fragments that include these sequences can be used to vaccinate directly.

25 In a further specific embodiment, T cell receptors, whole T cells or fragments of TCRs that contain VB17, VB14 or VB3 can be used to immunize an individual having a T cell mediated pathology to treat or prevent the disease. In a specific embodiment, rheumatoid arthritis can be so treated. The immune response generated in the individual can neutralize or kill T cells having VB17, VB14 or VB3 and, thus, prevent or treat the deleterious effects of such VB-bearing T cells. Moreover, to the extent that VB17, VB14 or VB3 is common to T cell receptors on pathogenic T cells

mediating other autoimmune diseases or autoimmune diseases in general, such vaccines can also be effective in ameliorating such other autoimmune diseases.

As used herein, "VB17" refers to a specific human β -chain variable region of three T cell receptors. VB17 has the following amino acid sequence: MSNQVLCCVLC FLGANTVDGGITQSPKYLFRKEGQNVTLSC EQNLNHDAMYWYRQDPGQGLRLIYYSQ IVNDFQKGDIAEGYSVSREKKESFPLTVTSAQKNPTAFYLCASS (SEQ ID No. 7).

"VB14" refers to a specific human β -chain variable regions of another TCR. VB14 has the following amino acid sequence: MGPQLLGYYVLCCLLGAGPLEAQVTQNPRYLITV TGKKLTVTCSQNMNHEYMSWYRQDPGLGLRQIYYSMNVEVTDKGDVPEGYKVS RKEK RNFPLILESPSPNQTSLYFCASS (SEQ ID No. 8).

"VB3" refers to a family of specific human β -chain variable region. Two members of the VB3 family have been identified as VB3.1 and VB3.2. VB3.1 has the following amino acid sequence: MGIRLLCRVAFCFLAVGLVDVKV TQSSRYLVKRTGEKVFLECVQDMDHENMFWYRQDPGLGLRLIYFSYDVKMKEKGDIP EGYSVSREKKERFSLILESASTNQTSMYLCASS (SEQ ID No. 9). VB3.2 has the following amino acid sequence: MGIRLLCRVAFC FLAVGLVDVKVTQSSRYLVKRTGEKVFLECVQDMDHENMFWYQ RQDPGLGLRLIYF

The hypervariable or junctional regions are useful for the vaccines of the present invention. Hypervariable regions useful in the present invention include CDR1, CDR2, CDR3 and CDR4. The amino acid sequences of the CDR1, CDR2 and CDR4 hypervariable regions for VB3, VB14 and VB17 are shown in Figure 1.

The CDR3, also known as the V(D)J region, is useful as a vaccine of the present invention since T cell immunity elicited by peptides corresponding to this region is expected to be highly specific for a particular antigen. Due to the recombination of the V, D and J region genes prior to maturation, the amino acid sequence across these regions is virtually unique to each T cell and its clones.

However, as a germ-line element, the CDR2 region is also useful in human diseases such as MS and in particular RA. In RA studies, the results indicate a limited number of VBs among the activated T cells infiltrating the synovial target tissue with only a few incidences of sequence homology in the β V DJ region. Thus, peptides corresponding to the CDR2 region are viable alternatives for use as vaccines of the present invention. For example, the CDR2 region of VB3, DPGLGLRLIYFSYDVKMKEKG (SEQ ID No.75), of VB14, DPGLGLRQIYYSMNVEVTDKG (SEQ ID No.76), or of VB17, DPGQGLRLIYYSQIVNKFQKG (SEQ ID No.77), can be used.

Modifications in these sequences that do not affect the ability of the receptor or an immunogenic fragment thereof to act as an immunogen to stimulate the desired immune response are contemplated and are included in the definition of TCR fragment. The variable region can be joined with any D and J segment of the TCR. Further, immunogenically representative fragments of VB3, VB14 and VB17 are also included in the definition of "VB3," "VB14" and "VB17," respectively.

By "substantially pure," it is meant that the TCR is substantially free of other biochemical moieties with which it is normally associated in nature. Such substantially pure TCRs or fragments thereof, for instance, can be synthesized, produced recombinantly by

means known to those skilled in the art. In addition, whole TCRs can be enzymatically treated to produce such fragments.

In another embodiment, vaccine peptides can
5 correspond to the V β regions that contain sequences of high homology which are conserved among pathogenic TCRs. These regions of conserved homology include the conventional CDRs, such as CDR1 and CDR2, which are common to T cells bearing the same V β , and also the
10 superantigen binding site, which can be common to pathogenic TCRs bearing different V β s. The superantigen binding site is also known to be in or around the CDR4 hypervariable region.

The vaccines of the present invention comprise
15 peptides of varying lengths corresponding to the TCR or immunogenic fragments thereof. The vaccine peptides can correspond to regions of the TCR which distinguish that TCR from other nonpathogenic TCRs. Such specific regions can, for example, be located within the various region(s)
20 of the respective TCR polypeptide chains, for example, a short sequence spanning the V(D)J junction, thus restricting the immune response solely to those T cells bearing this single determinant.

The vaccines are administered to a host
25 exhibiting or at risk of exhibiting an autoimmune response. Definite clinical diagnosis of a particular autoimmune disease warrants the administration of the relevant disease-specific TCR vaccines. Prophylactic applications are warranted in diseases where the
30 autoimmune mechanisms precede the onset of overt clinical disease (for example, Type I Diabetes). Thus, individuals with familial history of disease and predicted to be at risk by reliable prognostic indicators

could be treated prophylactically to interdict autoimmune mechanisms prior to their onset.

TCR peptides can be administered in many possible formulations, including pharmaceutically acceptable mediums. In the case of a short peptide, the peptide can be conjugated to a carrier, such as KLH, in order to increase its immunogenicity. The vaccine can include or be administered in conjunction with an adjuvant, of which several are known to those skilled in the art. After initial immunization with the vaccine, further boosters can be provided. The vaccines are administered by conventional methods, in dosages which are sufficient to elicit an immunological response. Such dosages can be easily determined by those skilled in the art.

Appropriate peptides to be used for immunization can be determined as follows. Disease-inducing T cell clones reactive with the target antigens are isolated from affected individuals. Such T cells are obtained preferably from the site of active autoaggressive activity such as a lesion in the case of pemphigus vulgaris, the central nervous system (CNS) in the case of multiple sclerosis or the synovial fluid or tissue in the case of rheumatoid arthritis. Alternatively, such T cells can be obtained from blood of affected individuals. The TCR genes from these autoaggressive T cells are then sequenced. Polypeptides corresponding to TCRs or portions thereof that are selectively represented among disease inducing T cells (relative to non-pathogenic T cells) can then be selected as vaccines and made and used as described above. An alternative method for isolating pathogenic T cells is provided by Albertini in PCT Publication No. WO88/10314, published on December 29, 1988.

Alternatively, the vaccines can comprise anti-idiotypic antibodies which are internal images of the peptides described above. Methods of making, selecting and administering such anti-idiotypic vaccines are well known in the art. See, for example, Eichmann, et al., CRC Critical Reviews in Immunology 7:193-227 (1987), which is incorporated herein by reference.

In a further aspect of the present invention, methods of preventing the proliferation of T cells associated with a T cell mediated pathology are also contemplated. Such methods include determining a T cell receptor binding partner according to the above methods and administering an effective amount of such binding partner in an appropriate form to prevent the proliferation of the T cells. The methods can be used, for example, to build a tolerance to self antigens as in the case of an autoimmune disease.

The present invention also relates to other methods of preventing or treating a T cell pathology by inhibiting the binding of a T cell receptor to its TCR binding partner in order to prevent the proliferation of T cells associated with the T cell pathology. Ligands that are reactive with the T cell receptor or its binding partner at binding sites that inhibit the T cell receptor attachment to the binding partner can be used. Such ligands can be, for example, antibodies having specificity for the T cell receptor or its binding partner.

The invention also provides a method of preventing or treating a T cell mediated pathology in an individual comprising cytotoxically or cytostatically treating a VB-containing T-cells, particularly VB3, VB14 and VB17, in the individual. The VB-containing T cells are treated with a cytotoxic or cytostatic agent that

selectively binds to the VB region of a T cell receptor that mediates a pathology, such as RA or MS for example. The agent can be an antibody attached to a radioactive or chemotherapeutic moiety. Such attachment and effective agents are well known in the art. See, for example, Harlow, E. and Lane, Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory (1988), which is incorporated herein by reference.

Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a T cell mediated autoimmune disease. The invention describes clonal infiltrates of activated VB3, VB14 and VB17 T cells in the synovium of rheumatoid arthritis patients. The presence of these T cells in the diseased tissue of most of patients examined, their clonality, and the cytotoxic activity of one such T cell for synovial adherent cells, demonstrate a central role for T cells bearing these VBs in the pathogenesis of RA.

Activated T cell populations in the synovial tissue of RA patients have been examined by analyzing T cell receptor (TCR) mRNAs isolated from IL-2 receptor positive (IL-2R+) synovial T cells. As described in Example X(C), TCR mRNAs were amplified using a polymerase chain reaction (PCR) protocol designed to amplify human TCR B-chain genes containing virtually any desired VB gene element. In this analysis, clonal VB17 rearrangements were found to be enriched in the IL2-R+ population, indicating that VB17 T cells are likely involved in the pathogenesis of RA. A CD4+, VB17 bearing T cell clone has been isolated from one of the synovial tissue specimens and its in vitro cytotoxicity for synovial adherent cells supports the direct involvement of VB17 T cells in RA.

Additional studies were conducted to ensure that the prevalence of VB17 T cells in the initial studies did not result from an amplification bias for the VB consensus primer, and to examine the involvement of other TCR VB gene families in RA. As described in Example XI, RNAs from activated (IL2-R+) synovial T cells were analyzed by PCR-amplification with a panel of VB-specific PCR primers. In this analysis, VB17 transcripts were found in four of the five patients tested, confirming the association of VB17 with RA and validating the utility of the VB consensus primer. In addition, VB14 was found in four of the five RA patient samples and VB3 and VB9 were detectable in three of five patients.

The sequences of these various VB polypeptides were examined for homology to VB17. The results are reported in Table 1.

TABLE 1
Relative Homologies of TCR B Chain Polypeptides
With Human VB17

20	hVB	100%	(94aa)
	mVB6	69.1%	(94aa)
	hVB3	58.5%	(94aa)
	hVB12.1	53.2%	(94aa)
	hVB14	52.1%	(94aa)
25	mVB7	51.7%	(89aa)
	hVB9	33.0%	(94aa)

hVB = human VB

mVB = mouse VB

As shown in Table 1, mouse VB6 is most closely homologous, followed by hVB3, hVB12.1, hVB14, and mVB7. Three of the human VBs, VB3, VB14 and VB17, were detected in the synovium of RA patients. VB12.1 was negligible in the synovium despite considerable overall homology with

VB17. In contrast, VB9 was found in three of five synovial samples, yet is only weakly homologous to VB17.

A surprising discovery is the greater homology found among all of the VBs detected in the synovia of RA patients, except VB9, in a contiguous stretch of 15 amino acids located carboxy to the CDR2 region. The 15 amino acid sequences of these VBs as well as other human and mouse VBs are shown in Table 2. Within the β -chain, this region of conservation corresponds positionally to that previously shown to contain superantigen binding sites.

TABLE 2

Proposed Superantigen Binding Site in
RA Associated VB Genes

			<u>%H¹</u>	<u>Seq. ID. No.</u>
15		+-		
	hVB17	EGYSVS <u>RE</u> KKESFPL		11
		+-		
	hVB3	EGYSVS <u>RE</u> KKERFSL	86.7	12
		+-		
20	hVB14	EGYKVS <u>RKE</u> KRNFL	66.7	13
	hVB12.1	DGYSVSRSKTEDFLL	66.7	14
	hVB9	NRFSPKSPDKAHLNL	<30	15
<hr/>				
		+-		
25	mVB6	EGYDAS <u>RE</u> KKSSFSL	73.3	16
		+-		
	mVB7	KGYRVSRKK <u>RE</u> HFSL	64.3	17
<hr/>				
30	mVB8.2a	DGYKASRPSQENFSL		18
		+-		
	mVB8.2c <u>KE</u>		19
	hVB13.2	DGYNVSRLLKKQNFLLGLE		20

35 ¹%H = % homology compared with VB17

The exogenous superantigen, SEC₂, stimulates human VB13.2 T cells as a result of binding to a site on VB13.2 as described in Choi et al., Nature 346:471-473 (1990), which is incorporated herein by reference. The sequence of this binding site is shown in Table 2 as the last 11 amino acids for VB13.2.

A binding site for Mls-1a, an endogenous superantigen, has also been mapped to this region as described in Pullen et al., Cell 61:1365-1374 (1990), which is incorporated herein by reference. Identification of this region as the Mls binding site involved the study of VB8.2a, the VB8.2 isoform common to laboratory mice, and VB8.2c, a β -chain found in wild mice. These β -chain polypeptides are distinguished functionally by their differential reactivities with Mls-1a and structurally by a difference of five amino acids. Of particular importance are the residues at position 70 and 71. The responder β -chain, VB8.2c, has lysine and glutamic acid, respectively, at these positions. Specific mutagenesis of the non-responder gene to encode a lysine-glutamic acid pair at positions 70 and 71 rendered that non-responder β -chain Mls-1a-reactive. This confirms the region as one of superantigen binding. Thus, both these exogenous and endogenous superantigens can bind in the vicinity of the 15 amino acid sequence homology identified in Table 2. In addition, the lysine-glutamic acid pair charge motif is implicated in Mls-1a reactivity. Involvement of this charge motif in Mls-1a reactivity is confirmed by its presence in mouse VB6 and VB7, two other Mls-1a reactive murine β -chains. The VB8.2c, VB6 and VB7 charge motif of a lysine or arginine followed by a glutamic acid residue is underlined in Table 2. Thus, the superantigen binding site for Mls-1a is characterized by this charge motif contained within the region of local homology.

The present invention is directed to the unexpected discovery that human VB3, VB14 and VB17 have a region that corresponds to the Mls-1a binding site. These three human VBs display a significant degree of overall homology within the entire 94 amino acid sequence and of local homology within the 15 amino acid sequence with mVB6 and mVB7. Each of these VBs possess a lysine or arginine/glutamic acid pair, which are underlined in Table 2 and represent what is meant by the term "charge motif." VB12.1, while displaying a high degree of overall and local homology with VB3, VB14 and VB17, lacks the charge motif, perhaps accounting for its presence in the synovium of only one of five RA patients. VB9 shows no overall or local homology to VBs 3, 14 or 17 and lacks the charge motif.

The presence of VB3, VB14 and VB17-bearing T cells has been demonstrated among the activated synovial T cells in RA. These three B-chain polypeptides, in contrast to other known VBs, possess overall and local sequence homology and an apparent superantigen binding charge motif. These results indicate that VB-specific T cell activation by superantigen plays a role in RA.

VB3, VB14 and VB17 are the only known human VB chains to possess this apparent superantigen binding site characterized by this local sequence homology and the identified charge motif. However, it is possible that other VBs may become known that contain such binding sites. Thus, a substantially pure VB3, VB14 or VB17 sequence containing the charge motif can be used as an immunogen in the vaccines of the present invention. For example, the sequences or fragments thereof shown in Table 2 for VB3, VB14 and VB17 can be used. Vaccines containing any combination of these three VB sequences, including all three sequences, can be used effectively to ameliorate T cell associated diseases.

In addition, other common V(D)J sequences of the β -chain observed in RA patients are listed in Table 3. The results taken from two different RA studies show sequence homologies in the BV(D)Js from four different clones, which indicates the usefulness of peptides corresponding to the CDR3 region as appropriate vaccine candidates.

TABLE 3

β -Chain VDJ Sequences Found
in Common in RA Patients

<u>Patient</u>	<u>VB</u>	<u>VDJ Sequence</u>	<u>Jβ</u>	<u>Seq. ID No.</u>
1012	VB14	A S S L G G A V S - Y N	JB2.1	3
1013	VB3	A S S L G G E E T Q Y F	JB2.5	4
C	VB3	A S S L G G F E T Q Y F	JB2.5	5
15 A	VB3	A S S L G G T E A - F F	JB1.1	6

As noted, the invention provides the discovery that specific variable regions of the β -chains of three TCRs, designated VB3, VB14, and VB17, are closely associated with T cell mediated pathologies, especially rheumatoid arthritis in human subjects. This discovery allows for the detection, prevention and treatment of rheumatoid arthritis using the methodology set out in this invention. Similar therapeutic approaches set out above for EAE can be applied to rheumatoid arthritis by those skilled in the art.

Specifically, the invention provides a method of diagnosing or predicting susceptibility to T cell mediated pathologies in an individual comprising detecting T cells having the β -chain variable region from VB3, VB14 or VB17 in a sample from the individual, the presence of abnormal levels of such VB-containing T cells

indicating the pathology or susceptibility to the pathology. The V β -containing T cells can be qualitatively or quantitatively compared to that of normal individuals. Such diagnosis can be performed, for example, by detecting a portion of the V β s that does not occur on non-rheumatoid arthritis associated β -chain variable region T-cell receptors. The V β s of the present invention can be detected, for example, by contacting the V β s with a detectable ligand capable of specifically binding to the individual V β s. Many such detectable ligands are known in the art, e.g. an enzyme linked antibody. Alternatively, nucleotide probes, complementary to the individual V β of interest, encoding nucleic acid sequences can be utilized to detect such V β -containing T cells, as taught, for instance, in Examples X and XI.

The invention also provides a method of preventing or treating a T cell mediated pathology comprising preventing the attachment of a V β 3-, V β 14- or V β 17-containing T-cell receptor to its binding partner. In one embodiment, attachment is prevented by binding a ligand to V β 3, V β 14 or V β 17. In an alternative embodiment, attachment is prevented by binding a ligand to the V β 3, V β 14 or V β 17 binding partner. Attachment can be prevented by known methods, e.g. binding an antibody to the individual V β s or to its binding partner in order to physically block attachment.

Multiple Sclerosis

T cells causative of multiple sclerosis (MS) have not previously been identified, though MBP-reactive T cells have been proposed to play a role due to the clinical and histologic similarities between MS and EAE. In rat and mouse models of EAE, MBP-reactive, encephalitogenic T cells show striking conservation of β -

chain V(D)J amino acid sequence, despite known differences in MHC restriction and MBP-peptide antigen specificity. One embodiment of the invention is premised on the observation that a human myelin basic protein (MBP)-reactive T cell line, derived from an MS patient, has a TCR β -chain with a V(D)J amino acid sequence of VB4 homologous with that of β -chains from MBP-reactive T cells mediating pathogenesis in experimental allergic encephalomyelitis (EAE), an animal model of MS, as shown in Table 4. This finding demonstrates the involvement of MBP-reactive T cells in the pathogenesis of MS and demonstrates that TCR peptides similar to those described herein for the prevention of EAE can be appropriate in treating MS. As shown in Table 4, a VDJ sequence of VB12, CAIGSNTE (SEQ ID No. 2) and one of VB4, SGDQGGNE (SEQ ID No. 1), have been observed in MS patients.

TABLE 4

β -Chain VDJ Sequences in MS Patients
Homologous to Rat VDJ Sequences

<u>VB</u>	<u>VDJ Sequence</u>	<u>SEQ ID Nos.</u>
RAT VB8.2	S S D S S N T E	21
RAT VB8.2	S S D S G N T E	22
HUMAN VB4	S G D Q G G N E	1
HUMAN VB12	C A I G S N T E	2

The activated cells of one MS patient from the CSF were analyzed and found to be predominantly VB14 and VB3. In both cases, there was a predominate clone of VB14 and a predominate clone of VB3. These findings indicate that the results from the RA studies relating to the same VBs can be extended to other autoimmune pathologies, including MS.

In this regard, the invention is directed to the discovery that β -chain VDJ fragments homologous to VDJ sequences found in rodent EAE, such as SGDQGGNE (SEQ ID No.1) and CAIGSNTE (SEQ ID No. 2), are closely associated with multiple sclerosis in human subjects. This discovery allows for the detection, prevention and treatment of multiple sclerosis using the methodology set out in this invention. Similar therapeutic approaches set out herein for EAE can be applied to multiple sclerosis by those skilled in the art.

Specifically, the invention provides a method of diagnosing or predicting susceptibility to multiple sclerosis in an individual comprising detecting T cells having various VBs, such as VB4 or VB12, and particularly having substantially the sequence SGDQGGNE (SEQ ID No.1) or CAIGSNTE (SEQ ID No.2), in a sample from the individual, the presence of the sequence indicating multiple sclerosis or susceptibility to multiple sclerosis. The sequences can be detected, for example, by contacting T cells or TCRs with a detectable ligand. Many such ligands are known in the art, for example, an enzyme linked or otherwise labeled antibody specific for the sequence. Alternatively, nucleotide probes complementary to the nucleic acid encoding the sequence can be utilized as taught, for instance, in Example IX.

The invention also provides a method of preventing or treating multiple sclerosis comprising preventing the attachment of a T-cell receptor containing various VBs, including VB4, VB12 or fragments thereof, such as those having substantially the SGDQGGNE (SEQ ID No.1) or CAIGSNTE (SEQ ID No.2) sequence to its binding partner. In one embodiment, attachment is prevented by binding a ligand to the sequence. In an alternative embodiment, attachment is prevented by binding a ligand to the binding partner. Attachment can be prevented by

known methods, such as binding an antibody to these VBs, and in particular to the SGDQGGNE (SEQ ID No.1) or CAIGSNTE (SEQ ID No.2) sequences, to physically block attachment.

5 The invention also provides a method of preventing or treating multiple sclerosis in an individual comprising cytotoxically or cytostatically treating T cells containing various VBs, including VB4, VB12 and fragments thereof, particularly those having
10 substantially the SGDQGGNE (SEQ ID No.1) or CAIGSNTE (SEQ ID No.2) sequence in the individual. In one embodiment, T-cells are treated with a cytotoxic or cytostatic agent which selectively binds to these VBs or their immunogenic fragments. The agent can be, for example, an antibody
15 attached to a radioactive or chemotherapeutic moiety.

T Cell Pathologies of Malignant Etiology

To illustrate the utility of TCR vaccination, autoimmune disease has been discussed. However, T cell lymphoma is another T cell pathology
20 which would be amenable to this type of treatment. Application of this technology in the treatment of T lymphoma would be conducted in virtually identical fashion. In one respect, however, this technology is more readily applied to T cell proliferative disease
25 since the isolation of the pathogenic T cells is more easily accomplished. Once the clones are isolated, the technology is applied in the manner described herein. Specifically, the TCR genes of the T lymphomas are sequenced, appropriate regions of those TCRs are
30 identified and used as vaccines. The vaccines can comprise single or multiple peptides, and can be administered in pharmaceutically acceptable formulations, with or without adjuvants, by conventional means.

Gene Therapy

The present invention further relates to an alternative method of treating or preventing a T cell mediated pathology by gene therapy. In this method, a nucleic acid encoding for a TCR or an immunogenic fragment thereof is first inserted into an appropriate delivery system, for example a plasmid. The nucleic acid can be DNA or RNA encoding for TCRs, immunogenic fragments thereof or anti-idiotypic antibodies that can be used as vaccines in the present invention. Such DNA or RNA can be isolated by standard methods known in the art. The isolated nucleic acid can then be inserted into a suitable vector by known methods. Such methods are described, for example, in Maniatis et al., Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Laboratory 1982), which is incorporated herein by reference.

The vector is subsequently administered directly into a tissue of an individual. Preferably, the DNA or RNA-containing vector is injected into the skeletal muscle of the individual. For example, a 1.5 cm incision can be made to expose the quadricep muscles of the subject. A 0.1 ml solution containing from 10-100 μ g of a DNA or RNA plasmid and 5-20% sucrose is injected over 1 minute into the exposed quadricep muscles about 0.2 cm deep. The skin is thereafter closed. The amount of DNA or RNA plasmid can range from 10 to 100 μ l of hypotonic, isotonic or hypertonic sucrose solutions or sucrose solutions containing 2 mM CaCl_2 . The plasmid containing solutions can also be administered over a longer period of time, for example, 20 minutes, by infusion. The in vivo expression of the desired gene can be tested by determining an increased production of the encoded polypeptide by the subject according to methods

known in the art or as described, for example, in Wolff et al., Science 247:1465-1468 (1990).

It is believed that the treated cells will respond to the direct injection of DNA or RNA by
5 expressing the encoded polypeptide for at least about 60 days. Thus, the desired TCR, immunogenic fragment or anti-idiotypic antibody can be effectively expressed by the cells of the individual as an alternative to vaccinating with such polypeptides.

10 The present invention also relates to vectors useful in the gene therapy methods and can be prepared by methods known in the art. Compositions containing such vectors and a pharmaceutically acceptable medium are also provided. The pharmaceutically acceptable medium should
15 not contain elements that would degrade the desired nucleic acids.

The following examples are intended to illustrate but not limit the invention.

EXAMPLE I

20

RAT MODEL OF EAE

Female Lewis rats, (Charles River Laboratories, Raleigh-Durham, NC) were immunized in each hind foot pad with 50 µg of guinea pig myelin basic protein emulsified in complete Freund's adjuvant. The first signs of
25 disease were typically observed 9-11 days post-immunization. Disease severity is scored on a three point scale as follows: 1=limp tail; 2=hind leg weakness; 3=hind leg paralysis. Following a disease course of approximately four to six days, most rats
30 spontaneously recovered and were refractory to subsequent EAE induction.

EXAMPLE II
SELECTION AND PREPARATION OF VACCINES

Vaccinations were conducted with a T cell receptor peptide whose sequence was deduced from the DNA sequence of a T cell receptor beta gene predominating among EAE-inducing T cells of B10.PL mice. The DNA sequence was that reported by Urban, et al., supra, which is incorporated herein by reference. A nine amino acid peptide, having the sequence of the VDJ junction of the TCR beta chain of the mouse, was synthesized by methods known to those skilled in the art. The sequence of this peptide is: SGDAGGGYE (SEQ ID No.23). (Amino acids are represented by the conventional single letter codes.) The equivalent sequence in the rat has been reported to be: SSD-SSNTE (SEQ ID No. 24) (Burns et al., J. Exp. Med. 169:27-39 (1989)). The peptide was desalted by Sephadex G-25 (Pharmacia Fine Chemicals, Piscataway, NJ) column chromatography in 0.1 M acetic acid and the solvent was subsequently removed by two cycles of lyophilization. A portion of the peptide was conjugated to keyhole limpet hemocyanin (KLH) with glutaraldehyde at a ratio of 7.5 mgs of peptide per mg of KLH. The resulting conjugate was dialyzed against phosphate buffered saline (PBS).

EXAMPLE III
VACCINATION AGAINST EAE

Vaccines used in these studies consisted of free VDJ peptide and also of VDJ peptide conjugated to KLH. These were dissolved in PBS and were emulsified with equal volumes of either (1) incomplete Freund's adjuvant (IFA) or (2) complete Freund's adjuvant (CFA) made by suspending 10 mg/ml heat killed desiccated Mycobacterium tuberculosis H37ra (Difco Laboratories, Detroit, MI) in IFA. Emulsions were administered to 8-12

week old female Lewis rats in a final volume of 100 microliters per animal (50 μ l in each of the hind footpads). 5 μ g of unconjugated VDJ peptide were administered per rat. KLH-VDJ conjugate was administered at a dose equivalent to 10 μ g of KLH per rat. Twenty-nine days later each rat was challenged with 50 μ g of guinea pig myelin basic protein in complete Freund's adjuvant in the front footpads. Animals were monitored daily beginning at day 9 for clinical signs of EAE and were scored as described above. The results are presented in Table 5. As can be seen, not only was there a reduced incidence of the disease in the vaccinated individuals, but in those which did contract the disease, the severity of the disease was reduced and/or the onset was delayed. The extent of protection varied with the vaccine formulation, those including CFA as the adjuvant demonstrating the greatest degree of protection.

TABLE 5

20	Animal No.	Vaccination (Adjuvant)	Days After Challenge									
			10	11	12	13	14	15	16	17	18	
25	1	VDJ (IFA)	-	-	2	3	3	3	-	-	-	
	2	"	-	-	1	3	3	3	2	-	-	
	3	"	-	-	-	3	3	3	2	-	-	
	4	VDJ (CFA)	-	-	-	-	1	1	1	-	-	
	5	"	-	-	-	-	-	-	-	-	-	
	6	"	-	-	-	1	3	3	3	2	-	
	7	KLH-VDJ (CFA)	-	-	-	1	3	2	-	-	-	
	8	"	-	-	-	-	1	1	1	1	-	
	9	"	-	-	-	-	-	-	-	-	-	
30	10	KLH-VDJ (IFA)	-	1	3	3	2	2	1	-	-	
	11	"	-	-	3	3	3	3	3	2	-	
	12	"	-	-	1	3	3	3	3	-	-	
	13	NONE	1	3	3	3	3	1	-	-	-	
35	14	"	-	1	3	3	3	1	-	-	-	
	15	"	1	3	3	3	1	-	-	-	-	

Scoring: - no signs
 1) limp tail
 2) hind leg weakness
 3) hind leg paralysis

EXAMPLE IVVaccination against EAE with Lewis Rat VDJ peptides

The VDJ peptide used in the previous examples was synthesized according to the sequence of TCR β chain molecules found on EAE-inducing T cells in B10.PL mice. In addition, peptides were synthesized and tested which correspond to sequences found on encephalitogenic T cells in Lewis rats. These VDJ sequences are homologous with that of B10.PL mice, but not identical. The rat peptides were synthesized according to the DNA sequences reported by Burns, et al. and Chluba, et al., Eur. J. Immunol. 19:279-284 (1989). The sequences of these peptides designated IR1, 2, 3 and 9b are shown below, aligned with the B10.PL mouse sequence used in Examples I through III (VDJ):

		<u>SEQ ID No.</u>
VDJ	S G D A G G Y E	23
IR1	C A S S D - S S N T E V F F G K	25
IR2	C A S S D - S G N T E V F F G K	26
20 IR3	C A S S D - S G N - V L Y F G E G S R	27
IR9b	A S S D - S S N T E	28

The preparation, administration and evaluation of these vaccines were conducted as described in Examples I through III with the following exceptions: 50 μ g of the individual VDJ peptides were incorporated into vaccine formulations containing CFA; neither vaccinations in IFA nor vaccinations with peptides conjugated to KLH were conducted. Control animals were untreated prior to MBP challenge as in Example III or were vaccinated with emulsions of PBS and CFA to assess the protective effect of adjuvant alone. The results are shown in Table 6 below.

TABLE 6

	Animal No.	Vaccination (Adjuvant)	Days After Challenge								
			10	11	12	13	14	15	16	17	18
5	1	None	-	1	2	3	3	2	-	-	-
	2	"	1	3	3	3	2	-	-	-	-
	3	"	-	2	3	3	3	1	-	-	-
	4	PBS-CFA	1	2	3	3	3	-	-	-	-
	5	"	1	2	3	3	3	-	-	-	-
10	6	"	-	2	3	3	3	-	-	-	-
	7	IR1 (50 µg)	-	-	-	2	1	-	-	-	-
	8	"	-	-	-	-	1	3	-	-	-
	9	"	-	-	-	1	1	1	1	-	-
15	10	IR2 (50µg)	-	-	1	3	3	3	-	-	-
	11	"	-	-	-	-	2	2	3	3	-
	12	"	-	-	-	-	1	-	-	-	-
	13	IR3 (50µg)	1	3	3	3	2	-	-	-	-
	14	"	-	-	2	3	3	-	-	-	-
20	15	"	-	-	-	-	-	-	-	-	-
	16	IR9b (50 µg)	-	-	-	-	-	-	-	-	-
	17	"	-	-	-	-	-	-	-	-	-
	18	"	-	-	-	-	-	-	-	-	-
	19	"	-	-	-	-	-	-	-	-	-
25	Scoring:	-	no signs								
		1)	limp tail								
		2)	hind leg weakness								
		3)	hind leg paralysis								

As shown in Table 6, disease in unvaccinated control animals was observed as early as day 10. Disease was characterized by severe paralysis and wasting, persisted for 4 to 6 days and spontaneously remitted. PBS-CFA vaccinated rats displayed disease courses virtually indistinguishable from those of unvaccinated controls. In contrast, delays in onset were observed in some of the IR1, 2 or 3 vaccinated animals and others showed both delayed onset as well as decreased severity and/or duration of disease. Overall, however, vaccinations with the rat VDJ peptides (IR1-3) were slightly less effective than those with the mouse VDJ peptide (Example III). Vaccination with IR9b, however, afforded complete protection in all four animals in which it was tested. Importantly, no histologic lesions characteristic of disease were found in any of the four

EXAMPLE VIComparison of VB8.2 Peptide Lengths

The results of vaccinations conducted with the rat VB8 peptide are similar to those observed with the mouse and rat IR1, 2 and 3 peptides. Delayed onset as well as decreased severity and duration of disease was observed in one animal. One animal was completely protected.

It has been found that a corresponding 21 amino acid sequence of VB8.2 (residues 39-59) provided less protection than IR7 as shown in Table 8. The 21 amino acid sequence of VB8.2 is DMGHGLRLIHYSYDVNSTEKG (SEQ ID No.30).

39

TABLE 8

Comparison of Efficacy of Two β -Chain CDR2 Peptides
in Protecting from EAE in the Lewis Rats (50)

Vaccination	<u>#With Disease #Tested</u>	<u>Mean Max Severity</u>	<u>Mean Onset Duration</u>	<u>#With Histology #Tested</u>	<u>Mean histology Score</u>	<u>Mean S.L. (14 days)</u>
Non	10/10	2.8	10.5 8.4	10/10	3.7	---
Control Peptide/CFA	9/10	2.8	11.5 8.1	9/9	2.6	1.0
V88.2 ₂₀ CFA	6/10	1.3	11.2 3.8	8/10	1.0	16.4
PBS/CFA	9/10	2.5	12.1 6.0	9/10	2.6	---
V88.2 ₂₁ CFA	6/6	3.0	12.0 6.7	6/6	3.3	6.3

Each peptide was used at 100 μ g doses and dissolved in saline prior to being emulsified in an equal volume of complete Freund's adjuvant (CFA). Animals were challenged after 42 days with 50 μ g of guinea pig myelin basic protein in CFA. The CFA contained 10 mg/ml of mycobacteria tuberculosis. Injections and evaluation of clinical signs and histology were performed as previously described. Other animals (five per group) were immunized with peptides in the same way and their splenocytes were removed after 14 days to test for lymphocyte proliferation as described in Olee et al., J. Neuroimmunol. 21:235-240 (1989). The sequences of the 20 amino acid peptide and the 21 amino acid peptide are designated in Table 8 as VB8.2₂₀ and VB8.2₂₁ respectively.

15

EXAMPLE VIIVaccination with J region peptides

A peptide was synthesized which corresponds to the J α gene segment, TA39, found among both rat and mouse encephalitogenic T cell receptors. The sequence of this peptide, designated IR5, is:

IR5 RFGAGTRLTVK (SEQ ID No.31)

The efficacy of the J α TA39 peptide was tested in the Lewis rat model of EAE (Example I) as described in Examples II and III. 50 μ g of peptide were tested in CFA. Vaccinations in IFA or with peptide-KLH conjugates were not conducted. The results of these studies are shown in Table 9.

- - -

TABLE 9

Animal No.	Vaccination (Adjuvant)	Days After Challenge												
		10	11	12	13	14	15	16	17	18	19	20		
5	1	IR5 (50 µg)	-	-	-	-	-	2	1	1	1	1	-	
	2	"	-	-	-	-	-	-	-	-	-	-	-	
	3	"	-	-	-	-	-	-	-	-	-	-	-	
10	Scoring:	-	no signs											
		1)	limp tail											
		2)	hind leg weakness											
		3)	hind leg paralysis											

The results of vaccinations conducted with the rat J α TA39 peptide are more effective than those observed with the mouse VDJ peptide or the V88 peptide. Two of three animals were totally protected and, in the third, disease onset was markedly delayed. Severity was also reduced in this animal though disease persisted for a normal course of 5 days. Importantly, the two animals which were completely protected showed no histologic evidence of T cell infiltration of the CNS. This result indicates that vaccinating with the J_αTA39 very efficiently induces a regulatory response directed at encephalitogenic T cells. Even sub-clinical signs of disease were abrogated.

EXAMPLE VIIIVaccination with mixtures of TCR peptides

Vaccinations were conducted with a mixture of TCR peptides. This mixture contained 50 µg of each of the peptides IR1, 2, 3 and 5 (the three rat VDJ peptides and the rat JαTA39 peptide).

The efficacy of this peptide mixture was tested in the Lewis rat model (Example I) as described in

Examples II and III. Peptides were tested in CFA. Vaccinations in IFA or with peptide-KLH conjugates were not conducted. The results of these studies are shown in Table 10.

5

TABLE 10

	Animal No.	Vaccination (Adjuvant)	Days After Challenge								
			10	11	12	13	14	15	16	17	18
10	4	IR1, 2, 3, 5	-	-	-	-	-	-	-	-	-
	5	(50 µg each)	-	-	-	-	-	-	-	-	-
	6	"	-	-	-	-	-	-	-	-	-
15	Scoring:		-	no signs							
		1)		limp tail							
		2)		hind leg weakness							
		3)		hind leg paralysis							

The results of vaccinations conducted with the rat JαTA39 and three VDJ peptides were as effective as those described for IR9b in Table 6. All three animals were totally protected. In addition to the absence of any clinical signs of EAE, two of these three animals were completely free of histological evidence of T cell infiltration into the CNS while the third showed only two small foci of lymphocytic infiltration at the base of the spinal cord.

EXAMPLE IXMultiple Sclerosis VaccineA. Human MBP-reactive T cells

MBP-reactive T cell lines were established from peripheral blood mononuclear cells (PBMC) of nine chronic progressive MS patients and two healthy controls. Cells were maintained in culture by regular stimulation with purified human MBP and irradiated-autologous PBMC for

three days followed by four days in IL-2 containing medium.

B. PCR Amplification of TCR β -chain genes from MBP-reactive T cell lines

- 5 T cells were harvested from log phase cultures and RNA was prepared, amplified with the VB16mer primer and nested C β primers for 55 cycles as described in Example X.

C. TCR β -chain sequences of human MBP-reactive T cells

- 10 VB16mer amplified TCR β -chain genes from human MBP-reactive T cell lines were sequenced using the C β seq primer. Amplification products were gel purified, base denatured and sequenced from the C β seq primer. Readable DNA sequence was obtained from 5 of these lines,
15 indicating that predominant T cell clones had been selected by long term in vitro passage. One of these sequences, from the MS-Re cell line (Table 11), possessed a β -chain VDJ amino acid sequence that shared five of the first six and six of nine total residues with the β -chain
20 VDJ amino acid sequence conserved among MBP reactive, encephalitogenic T cells in the B10.PL mouse model of EAE. This sequence was not present among the predominant TCR rearrangements found in the remaining four human MBP reactive T cell lines.
- 25 To determine if similar sequences were present in the β -chain repertoire of the MBP-reactive T cell lines from other MS patients, PCR amplification was conducted with a degenerate (n=1024) 21-nucleotide primer (VBRe) corresponding to seven amino acids of this
30 sequence. RNAs were reversed transcribed and amplified in 20 cycle stage I reactions with the VB16mer and C β ext primers. One μ l aliquots of these stage I reactions were

reamplified for 35 cycles with the VBRe and CB int primers. One μ l aliquots of these reactions were analyzed by Southern blot hybridization with a 32 P-labeled human CB probe. This analysis revealed the 300 bp amplified product in the Re cell line and in one of the other MS patient lines, but not in MBP-reactive T cells from control subjects or in non-MBP reactive human T cell lines and clones. The presence of this sequence in two of the nine MS patient lines tested is compelling. Since this sequence is known to be conserved among encephalitogenic T cells in EAE, its detection among MBP-reactive T cells from MS patients demonstrates a role for T cells bearing this determinant in the pathogenesis of MS.

Immunogenic peptides having the sequence SGDQGGNE (SEQ ID No.1) can be synthesized as shown in Example II and used to immunize human subjects by methods demonstrated in Example III. Such immunizations can result in an effective immune response.

TABLE 11

A)	Sample	VB	DB	JB	SEQ ID No.
25	MS-Re	VB4.2 ctctgc	agcggagaccagggcggc S G D Q G G -	JB2.1 aatgagcagttcttc N E Q F F	32 33
23	B10.PL	S G D A G G G Y E			
30	B)	5' G G ^A _C G A ^C C A ^A G G ^A _C G G ^A _C A A ^C G A ^A _G 3' _G _T _G _T _T _T			34 35 36 37

EXAMPLE XDetection of Clonal Infiltrates of
Activated VB17 T Cells in the Synovium of
Rheumatoid Arthritis Patients5 A. T cell preparations from synovial tissue

Synovial tissue specimens were obtained from radiographically proven rheumatoid arthritis patients undergoing joint replacement therapy. Activated T cells were selected using magnetic beads and antibodies

10 reactive with the human IL2-R (α IL2-R) as follows. Synovial tissue was digested for 4 hrs at 37°C in RPMI + 10% Fetal Bovine Serum (FBS) containing 4 mg/ml collagenase (Worthington Biochemical, Freehold, NJ) and 0.15 mg/ml DNase (Sigma, St. Louis, MO.). Digests were

15 passed through an 80-mesh screen and single cells were collected by Ficoll density gradient centrifugation. Cells at the interface were washed and were incubated at 10⁶/ml for 30 min at 0°C with 5 μ g/ml control mouse IgG (Coulter Immunology, Hialeah, FL) in PBS containing 2%

20 FBS (PBS-FBS). Cells were washed three times and incubated for 30 min at 0°C with magnetic beads conjugated to goat anti-mouse IgG (Advanced Magnetix, Cambridge, MA). Beads were magnetically separated and washed three times with PBS-FBS. This preselection with mouse IgG

25 (mIgG) and magnetic beads was used to control for non-specific adsorption of T cells. The cells remaining in the initial suspension were further incubated 30 minutes at 0°C with 5 μ g/ml monoclonal mouse IgG reactive with the human T cell IL2-R (Coulter Immunology, Hialeah, FL).

30 Cells were washed and selected with magnetic beads as above. Beads from the IgG preadsorption and the IL2-R antibody selection were immediately resuspended in acidified-guanidinium-phenol-chloroform and RNA prepared as described in Chonezynski and Sacchi, Anal. Biochem.

162:156 (1987), which is incorporated herein by reference. Since RNAs were prepared without in vitro culture of the cells and the accompanying bias that may be induced, they are expected to accurately reflect T cell distributions in synovial tissue at the time of surgical removal. Only half of the mIgG and α IL2-R beads from patient 1012 were immediately processed for RNA. The remainder were cultured for 5 days in RPMI 1640, 5% FBS, 20% HL-1 (Ventrex Laboratories Inc., Portland, ME), 25mM HEPES, glutamine, antibiotics and 20% LAK supernatant (Allegretta et al., Science, 247:718 (1990)), which is incorporated by reference herein, as a source of IL-2. RNA was extracted from cultures of the α IL2-R beads (1012IL2.d5), but not from the 1012mIgG sample as no viable cells were present at the end of the 5 day culture.

A T cell clone was derived from the Ficoll pellet of patient 1008. The cells in the pellet were cultured at 2×10^6 /ml in media without IL-2 for two weeks. Non-adherent cells from this culture were cloned by limiting dilution onto autologous synovial cell monolayers. A CD4+ T cell clone 1008.8 was obtained and adapted to culture by regular stimulation with autologous synovial monolayers for 3 days in media without IL-2 followed by a 4 day culture in medium with LAK supernatant.

B. Lysis of Synovial Adherent Cells by 1008.8

Lysis of synovial adherent cells by 1008.8 was demonstrated as follows. Synovial cell monolayers were labeled as described in Stedman and Campbell, J. Immunol. Meth. 119:291 (1989), which is incorporated herein by reference, with 35 S for use as targets in CTL assays. Cells were trypsinized, washed and plated at 2000 cells per well of a 96-well round bottom microtiter plate.

1008.8 cells, cultured for 3 days prior to the assay with synovial adherent cells and medium containing LAK supernatant, were added to the targets at the indicated effector:target ratios. Cultures were incubated overnight at 37°C, centrifuged at 300xg for 2 minutes and radioactivity in 50 µl of the supernatant quantified. Per cent specific lysis was calculated relative to detergent-lysed targets by standard formulas. This clone is cytotoxic for synovial adherent cell targets in CTL assays (Table 12).

TABLE 12

<u>Effector:Target Ratio</u>	<u>% Specific Lysis</u>
5:1	7
10:1	16
25:1	32

C. PCR Amplification of TCR β-chain genes

TCR β-chain genes were amplified with several combinations of the primers shown in Figure 2. The VB16mer primer is a degenerate VB primer (n=256) which is predicted to bind 85% of human TCR β-chain genes at all 16 residues and 95% at 15 residues. This primer has been used to amplify TCR β-chains from more than 25 different human T cell clones, lines or primary tissue preparations. A spectrum of VB genes has been sequenced from these amplified DNAs, arguing against a significant bias of the primer for certain VB families. Thus, PCR amplification with the VB16mer primer facilitates analysis of T cell populations for which a priori knowledge of VB gene usage is unavailable.

T cell receptor β-chain genes were amplified in two-stage amplification reactions with nested pairs of

the primers shown in Figure 2. The primer sequences used in the polymerase chain reactions are listed in Table 13.

TABLE 13

5	VBcons	G AC CAAA	(SEQ ID NO. 47)
		5' T TC TGGTA CA 3'	(SEQ ID NO. 48)
		T TT TCGT	(SEQ ID NO. 49)
	VB17	5' TCACAGATAGTAAATGACTTTCAG 3'	(SEQ ID NO. 50)
	VB8	5' TCTCCACTCTGAAGATCC 3'	(SEQ ID NO. 51)
	VB12	5' GATTTCCTCCTCACTCTG 3'	(SEQ ID NO. 52)
10	5'CB	5' CAAGCTGTTCCCACCCGA 3'	(SEQ ID NO. 53)
	CBext	5' CCAGAAGGTGGCCGAGAC 3'	(SEQ ID NO. 54)
	CBint	5' GCGGCTGCTCAGGCAGTA 3'	(SEQ ID NO. 55)
	CBseq	5' CGACCTCGGGTGGGAACA 3'	(SEQ ID NO. 56)

15 RNAs were reverse transcribed for 1 hour at 42°C with 40pmol of the CBext primer in a 12 µl reaction using conditions described by Hart et al., The Lancet, p. 596 (1988), included by reference herein. Reactions were diluted with a master mix containing 40 pmols of the VB16mer primer, nucleotides and reaction buffer as above

20 but without MgCl₂ to give a final Mg⁺² concentration of 3.6 mM. Samples were denatured for 15 minutes at 95°C, 1 unit of heat stable recombinant DNA polymerase (Cetus Corporation, Emeryville, CA, Ampli-taq™) was added and 20 cycles of PCR conducted. Each cycle consisted of a 1 min

25 denaturation at 95°C, a two minute annealing step and a two minute extension at 72°C. The first two cycles were annealed at 37°C and 45°C, respectively, and the remainder at 50°C. One microliter aliquots of these stage I reactions were added to 100 µl stage II amplification

30 reactions (Cetus, Gene-Amp Kit™) containing 100 pmols of the CBint primer and 100 pmols of the VB8, VB17 or 5'CB primers or 700 pmols of the VB16mer primer. Stage II

amplifications were conducted as above with a 50°C annealing temperature and without the 37°C and 45 C ramping.

RNA samples from 1012IL2.d5 and 1008.8 cultures were amplified with the VB16mer and CBext primers in stage I reactions and with the VB16mer and the CBint primer in 35 cycle stage II reactions. Reaction products, purified from low melting agarose gel slices with Gene Clean glass beads (Bio 1D1, San Diego, CA), were base denatured and sequenced from the CBseq primer with T7 polymerase (Sequenase, United States Biochem, Cleveland, OH). A predominate VB sequence, corresponding to a single VB17 rearrangement (Table 14), was clearly readable in the 1012IL2.d5 sample. Other, less frequent rearrangements were detected as faint, uninterpretable background bands in the sequencing gels. Culture of these 1012.IL2 beads in IL2-containing medium without added accessory cells or antigen is not expected to induce de novo activation of T cells. Thus, the predominance of a single VB17 rearrangement in this sample reflects in vivo clonal expansion of VB17+ T cells in this patient. DNA sequence determination of TCR β -chain DNA amplified from the cytotoxic T cell clone, 1008.8, also revealed a VB17 rearrangement (Table 14). The presence of VB17 rearrangements in these two different types of synovial T cell samples, derived from two separate RA patients, implicates VB17 bearing T cells in the pathogenesis of RA.

50

TABLE 14

	Sample	VB	DB	JB	SEQ ID No.
5	1012	Y L C A S	K N P T V S	Y G Y T F	38
	day 5	tatctctgtgccagt	aaaaatcccacggtctcc	tatggctacaccttc	39
		VB17		JB1.2	
		Y L C A S	D N E S	F F G Q G	40
	1008.8	tatctctgtgccagt	gacaacgagagt	ttctttggacaaggc	41
10		VB17		JB1.1	
	1014	Y L C A S	V R D R R	N Y G Y T	42
	IL-2	tatctctgtgccagt	gtgaggacaggaga	aactatggctacacc	43
		VB17		JB1.2	
	1015	Y L C A S S	S I D S	S Y E Q Y	44
15	IL-2	tatctctgtgccagtagt	agtatagactcc	tcctacgagcagtagt	45
		VB17		JB2.7	

To determine whether or not VB17 rearrangements were present in the other magnetic bead RNA preparations, TCR β -chain genes were amplified with a VB17-specific primer in the second stage amplification after an initial amplification with the VB16mer. VB17 TCR DNA could be amplified from magnetic bead samples derived from the 4 patients examined. Ethidium bromide staining of electrophoresed reaction products revealed greater VB17 amplification in some of the IL-2R+ samples than in the corresponding controls. Accordingly, the relative amounts of VB17 TCRs in each control and IL-2R+ sample were quantified by slot blot hybridization analysis as follows.

30 RNAs from magnetic bead preps were amplified in the first stage with the VB16mer and C β ext primers and

then reamplified for twenty cycles with the CBint primer and each of the VB17, B88 and 5'CB primers.

Amplification reactions were serially diluted in 20X SSC, denatured by boiling and chilled in an ice slurry.

- 5 Samples were loaded onto nitrocellulose membranes, hybridized to a human TCR β -chain constant region probe and washed with 0.1X SSC, 0.1% SDS at 56°C. Bound radioactivity was quantified by liquid scintillation spectroscopy and endpoint dilutions were those samples
- 10 with fewer than 200 cpm bound. The amounts of product produced by forty total cycles with each of the respective primer combinations falls in the linear portion of a product versus cycle number quantification curve.

- 15 Amplifications with the 5'CB and CBint primer pair were used to estimate the total β -chain amplified from each sample, providing benchmarks for normalizing the results of VB17 and VB8 quantification in the respective IL-2R+ and control sample pairs (Table 15).
- 20 The quantity of VB17 DNA amplified was increased in the IL-2R+ samples, relative to the control samples, in 3 of the 4 patients. The magnitude of the increase ranged from 5-fold in patient 1015 to 40-fold in patient 1014 (Table 15). This enrichment was not a product of the
- 25 isolation procedure, since the quantity of VB8 DNA amplified was increased in the IL-2R+ fraction only in patient 1015.

TABLE 15

		<u>Endpoint Dilution</u>						
	Sample	CB	VB17	VB8	VB17/CB	VB17IL-2R mIgG	VB8/CB	VB8IL-2 mIgG
5								
	1	3,125	3,125	625	1		0.2	
	2	3,125	125	625	0.04	<u>25</u>	0.2	1
10	3	15,625	25	625	0.001		0.04	
	4	3,125	25	3,125	0.008	0.12	1	0.04
	5	15,625	625	125	0.04		0.008	
15	6	3,125	5	625	0.001	<u>40</u>	0.2	0.04
	7	15,625	625	15,625	0.04		1	
	8	78,125	625	3,125	0.008	<u>5</u>	0.04	<u>25</u> *
20	Sample 1 = 1012 IL-2R+, Sample 2 = 1012 mIgG, Sample 3 = 1013 IL-2R+, Sample 4 = 1013 mIgG, Sample 5 = 1014 IL-2R+, Sample 6 = 1014 mIgG, Sample 7 = 1015 IL-2R+, Sample 8 = 1015 mIgG.							
25	*In a follow-up study in which VB8 was sequenced, it was found that the enrichment of this VB was an artifact and that the value is ≤ 1 .							

VB17 rearrangements from the IL-2R+ RNAs of the three patients showing enrichment were amplified with the VB17 and CBint primer pair and the reaction products sequenced with the CBseq primer. As was shown for sample 1012 IL-2.d5, 1014 and 1015 contained single sequences (Table 14), indicative of clonal expansion of VB17 T cells *in vivo*. In contrast, direct sequencing of the rearrangements amplified with the VB8 specific primer was not possible due to significant heterogeneity in the β -chain product.

D. HLA-DR Analysis in Rheumatoid Arthritis Patients

HLA-DR analysis in rheumatoid arthritis patients was performed as follows. DNA from each patient was prepared by boiling 10^5 synovial cells in 200 μ l dH_2O .
5 Ten μ l were amplified for 35 cycles in a 100 μ l reaction (Cetus, Gene Amp KitTM) containing 100 pmols of each of the DR β PCR primers shown in Table 16 as DR β 1 and DR β 3. One-tenth μ l of this reaction was reamplified in 10 μ ls containing only the DR β 2 primer and 17 pmol of $\alpha^{32}P$ -dCTP
10 as the sole source of dCTP for 10 cycles. Reactions were spiked with 200 μ M dCTP and chased for 2 cycles. The resulting negative strand probes were hybridized to slot blots containing 10 pmol of the HLA-DR allele specific oligos (positive strands) using conditions previously
15 described by Amar et al., J. Immunol. 138:1947 (1987), which is incorporated herein by reference. The slots were washed twice for 20 minutes with tetramethylammoniumchloride (Wood et al., Proc. Natl. Acad. Sci. USA 82:1585 (1985)) which is incorporated
20 herein by reference) at 65-68°C and exposed to X-ray film.

Each of the patients in this study possessed at least one allele of the HLA-DR genes, DR4w4, DR1, DR4w14 or DR4w15, that are known to predispose for RA (Table
25 16). Also shown in Table 16 are HLA-DR allele specific oligonucleotides.

TABLE 16

								SEQ ID NO.
5				A				57
	DRB1	5'	G	A	G	T	C T G G A A C A G C	58
				C				59
					A			60
	DRB2	5'	G	T	A	G T T G	T T C T G C A	61
10					G			62

HLA-DR ALLELE-SPECIFIC OLIGONUCLEOTIDES

DRβ1 Genes

15	DR1, DR4w14, DR4w15	5'	CTC	CTC	GAG	CAG	AGG	CGG	GCC	GCG	3'	63
	DR2*	5'	T--	---	---	G-C	---	--C	---	---	3'	64
	DR3	5'	---	---	---	---	-A-	---	-G-	CG-	3'	65
	DR4w4	5'	---	---	---	---	-A-	---	---	---	3'	66
	DR4w13	5'	---	---	---	---	---	---	---	-A-	3'	67
20	DR5, DR6, DR4w10	5'	A--	---	--A	G-C	GA-	---	---	---	3'	68
	DR7	5'	A--	---	---	G-C	---	---	-G-	CA-	3'	69
	DR8	5'	T--	---	--A	G-C	---	---	---	CT-	3'	70
	DR9	5'	T--	---	---	-G-	---	---	---	-A-	3'	71

DRB3 Genes

	DR2	5'	A--	---	---	---	GC-	---	---	---	3'	72	
	DR3	5'	---	---	---	---	-A-	---	-G-	CAG	3'	73	
25	DR7, DR9	5'	---	---	---	---	-G-	---	---	---	-A-	3'	74

	<u>Patient</u>	<u>HLA-DR</u>
30	1008	1,4w4
	1012	1,3
	1013	1,7
	1014	1,4w4
	1015	4w4,4w4

* The dashes refer to amino acids in common with DR1, DR4w14, DR4w15

35 T cell receptors containing V β 17 or fragments thereof which are immunogenic or can be made immunogenic can be used to immunize human subjects by methods

demonstrated by Example VIII. Such immunizations can result in an effective immune response.

EXAMPLE XI

Synovial tissue specimens were obtained from
5 proven RA patients undergoing joint replacement surgery.
HLA-DR analysis was conducted as described in Example
IX(D).

A. Polymerase chain reaction (PCR) amplification of T cell receptor β -chain genes

10 T cell receptor β -chain genes were amplified in
two-stage amplification reactions with nested pairs of
HPLC-purified oligonucleotide primers (Midland Certified
Reagents, Midland, TX.) shown in Figure 3. RNAs were
reverse transcribed (1 hour, 42°C) with the CBext primer
15 (40 pmol) in 12 μ l of reaction buffer (Hart et al.,
Lancet ii:596-599 (1988)). Reactions were diluted with a
master mix (8 μ l) containing the VBcons primer (40 pmol),
nucleotides and reaction buffer minus $MgCl_2$ (final Mg^{+2}
concentration= 3.6 mM). Samples were denatured (15
20 minutes, 95°C) and 20 cycles of PCR were conducted using
Taq polymerase (1 unit, Cetus Ampli-taq). Each cycle
consisted of a 1 minute denaturation at 95°C, a two minute
annealing step and a two minute extension at 72°C. The
first two cycles were annealed at 37°C and 45°C and the
25 remainder at 50°C. One microliter aliquots of these Stage
I reactions were added to 100 μ l Stage II amplification
reactions (Cetus Gene-Amp Kit) containing the CBint
primer (100 pmol) and either the VB8, VB12, VB17, or 5'CB
primers (100 pmol) or the VBcons primer (100-700 pmol).
30 Stage II amplifications were conducted for the indicated
number of cycles with a 50°C annealing temperature and
without the 37°C and 45°C ramping.

B. VB17 T cells are cytotoxic for synovial adherent cells

Two parallel cultures were established from single cell suspensions, derived by enzymatic digestion as described below from the synovial tissue of patient 1008. The first, a bulk culture of total synovial cells, was plated at 2×10^6 /ml in RPMI 1640, 5% FBS, 20% HL-1 (Ventrex Laboratories, Portland, ME), 25mM HEPES, glutamine and antibiotics. The culture was grown undisturbed for two weeks in the absence of exogenous antigen or growth factors. The second, a synovial cell monolayer culture, was initiated as above and regularly trypsinized and passaged during this two-week period. Monolayer cells were plated at 5×10^4 cells/well in flat-bottomed, 96-well microtiter plates and cultured overnight. Non-adherent cells from the total synovial cell culture were then plated at 10 cells/well onto the monolayers. Wells positive for T cell growth were expanded and adapted to culture by regular stimulation with autologous synovial monolayers for 3 days in media without IL-2 followed by a 4 day culture in medium containing 20% supernatant from lymphokine activated killer (LAK) cells, a source of IL-2 (Allegretta et al., Science 247:718-721 (1990)). Later passages were adapted to weekly stimulation with allogeneic PBLs and anti-CD3 antibody (Coulter Immunology, Hialeah, FL) in place of synovial cell monolayers.

Cytotoxicity assays were conducted as described Stedman et al., Immunol. Meth. 119:291-294 (1989), incorporated herein by reference, using ^{35}S -labeled synovial monolayer cells or EBV-transformed B cells as targets. Target cells were labeled overnight, trypsinized (adherent cells only), washed and plated at 2000 cells per well in 96-well round bottom microtiter plates. T cells were activated with allogenic PBLs and anti-CD3 antibody in medium containing LAK supernatant

for 7 days prior to the assay and added to the targets at the indicated effector:target ratios. Cultures were incubated overnight at 37°C, centrifuged at 300xg for 2 minutes and radioactivity in 50 µl of the supernatant
5 quantified.

The per cent specific lysis was calculated relative to detergent-lysed targets as described in Townsend et al., Cell 44:959-968 (1986), incorporated herein by reference. At effector:target ratios of 1.0,
10 2.5 and 5.0, the percent lysis of synovial adherent cells by 1008.8 T cells was approximately 4%, 14% and 33%, respectively. By comparison, at the same effector:target ratios, 1008.8 T cells had no demonstrated effect on EBV-transformed B cell targets. Similarly, at the same
15 effector:target ratios, the percent lysis of synovial adherent cells by MS3 cells was approximately 0%, 0% and 3%, respectively.

T cells were isolated from the synovial tissue of patient 1008 by co-cultivation with synovial cell
20 monolayers. Since the antigens recognized by pathogenic T cells in RA are unknown, synovial cell monolayers were used as stimulators of in vitro synovial T cell growth. The relevant target cells were assumed to be present in a bulk adherent cell culture from diseased synovium. Of
25 192 microwell co-cultures plated, 7 were positive for T cell growth within 10-14 days. T cells were expanded and maintained in vitro by alternately stimulating with synovial cell monolayers and medium containing LAK supernatant. Flow cytometry revealed that each of these
30 seven cultures was 100% CD4+. One culture designated 1008.8, grew especially vigorously and, as assessed microscopically promoted the destruction of the monolayer cells. CTL assays confirmed the cytotoxicity of 1008.8 for these monolayer targets as discussed above.
35 Cytolysis was specific for synovial cell targets, as no

lysis of autologous Epstein-Barr virus-transformed B cells was demonstrable. Neither of the targets was lysed by a CD4+, myelin basic protein-reactive human CTL clone, MS3, excluding the possibility that the monolayer cells were susceptible to lysis by any activated T cell. In repeated assays with 1008.8, specific lysis never exceeded 30-35%, suggesting that the relevant synovial target cell comprises approximately that proportion of the total monolayer culture, which based on morphology is a mixture of multiple cell types.

The T cell receptor (TCR) β -chain gene of 1008.8 was amplified by the polymerase chain reaction (PCR) as described in Mullis et al., Meth. Enzym. 155:335-350 (1987), incorporated herein by reference, and its DNA sequence was determined. Amplification was accomplished using a consensus V β primer (V β cons), a degenerate 16 nucleotide primer (n=256) which is homologous at all 16 residues with 78%, and at 15 of 16 residues with 98%, of known human TCR V β genes, which have been compiled by Kimura et al., J. Immunol. 17:375-383 (1987). This primer was designed to amplify β -chain rearrangements containing any of the known V β genes, thus allowing analysis of T cell clones for which a priori knowledge of V β gene usage is unavailable. Sequencing of the V β cons-amplified β -chain gene of 1008.8 revealed a single V β 17-J β 1.1 rearrangement as shown in Table 16. Subsequently, the remaining six cultures derived from patient 1008 were analyzed by PCR amplification using a V β 17-specific primer shown in Table 13. TCR genes from three of those six were amplifiable, indicating that, of the 7 T cell cultures obtained from the synovium of this patient, 4 had rearranged and expressed the V β 17 gene.

C. VB17 T cells are enriched among activated T cells in RA synovium

Synovial tissue was digested with agitation for 4 hrs at 37°C in RPMI-1640 and 10% fetal bovine serum (FBS) containing 4 mg/ml collagenase (Worthington Biochemicals, Freehold, NJ) and 0.15 mg/ml DNase (Sigma Chemical, St. Louis, MO). Digests were passed through an 80-mesh screen and single cells were collected from the interface of Ficoll density gradients, washed and incubated at 10^6 /ml for 30 min at 0°C with 5 µg/ml control mouse IgG (Coulter) in PBS containing 2% BSA. Cells were washed 3X and incubated for 30 min at 0°C with magnetic beads conjugated to goat anti-mouse IgG (Advanced Magnetix, Cambridge, MA). After magnetic removal of the beads, the remaining cells were incubated 30 minutes at 0°C with 5 µg/ml mouse anti-human IL-2R (Coulter), washed and selected with magnetic beads as above. Cell-coated beads from the mIgG preadsorption and the IL-2R antibody selection were washed 3X, immediately resuspended in acidified-guanidinium-phenol-chloroform and RNA was prepared as described in Chomczynski et al., Anal. Biochem. 162:156-159 (1987).

RNAs from magnetic bead preparations were reverse transcribed and amplified for 20 cycles in stage I reactions with the VBcons and CBext primers. One µl of each reaction was reamplified for 20 cycles in individual stage II reactions containing the CBint primer in conjunction with the VB17, VB8, VB12 and 5'CB primers. Aliquots of each reaction were diluted in 20X SSC, denatured by boiling and chilled in an ice slurry. Samples were loaded onto nitrocellulose membranes, hybridized to a human TCR β-chain constant region probe and washed with 0.1X SSC, 0.1% SDS at 56°C. Bound radioactivity was quantified by liquid scintillation spectroscopy. The amounts of product produced by 40 total cycles with each of the respective primer

combinations falls in the linear portion of a product versus cycle number quantification curve. Values shown in Table 17 below reflect the relative increase or decrease for the specific VBs in the IL2-R+ versus mIgG controls calculated according to the formula:

$$\frac{\text{specific VB cpms (IL2-R+)}}{\text{specific VB cpms (mIgG)}} \div \frac{\text{CB cpms (IL2-R+)}}{\text{CB cpms (mIgG)}}$$

TABLE 17

		Ratio <u>IL-2R+</u> <u>mIgG</u>		
10	<u>Sample</u> <u>Experiment #</u>	<u>VB17</u>	<u>VB8</u>	<u>VB12</u>
1012				
	1	1.88	0.42	1.35
	2	1.60	0.39	0.72
15	3	<u>2.11</u>	<u>0.48</u>	<u>0.64</u>
	X ⁻ ±S.D.	1.86 ±0.25	0.43 ±0.04	0.900 ±0.38
1013				
	1	2.54	0.49	N.D.
	2	3.65	0.87	0.87
20	3	<u>4.29</u>	<u>2.07</u>	<u>0.96</u>
	X ⁻ ±S.D.	3.49 ±0.88	1.14 ±0.82	0.91 ±0.06
1014				
	1	4.70	0.17	N.D.
	2	1.68	0.10	1.50
25	3	1.92	0.09	0.44
	4	<u>2.34</u>	<u>0.07</u>	<u>0.55</u>
	X ⁻ ±S.D.	2.66 ±1.38	0.10 ±0.04	0.83 ±0.58
1015				
	1	3.40	0.20	0.85
30	2	<u>2.85</u>	<u>0.47</u>	<u>1.82</u>
	X ⁻ ±S.D.	3.12 ±0.50	0.38 ±0.15	1.33 ±0.68

Next, the presence of VB17 T cells in the synovial tissue of other RA patients was determined. Since the rheumatoid synovium contains a mixture of activated and non-activated T cells, the activated T
5 cells were identified as the most relevant for the initiation and perpetuation of the disease pathogenesis. Thus, activated T cells from single-cell suspensions of synovial tissue were selected using magnetic beads and antibodies reactive with the human interleukin-2 receptor
10 (IL-2R). Cell suspensions from each patient were pretreated with an isotype-matched mouse IgG (mIgG) and magnetic beads to control for non-specific adsorption. RNAs were directly extracted from cells in the IL-2R+ and control samples without in vitro culture and, therefore,
15 are expected to accurately reflect T cell distributions in synovial tissue at the time of surgical removal.

The initial PCR amplifications of these magnetic bead RNA preparations revealed greater amounts of VB17 PCR product in the IL2-R+ samples than in the
20 corresponding mIgG controls. The presence of TCR mRNA in the mIgG samples indicates that T cells non-specifically adhered to the magnetic beads. The apparent increase in the IL2-R+ sample suggests that the activated T cell compartment contained more VB17 T cells than the
25 unselected synovial T cell compartment. Thus, a quantitative PCR analysis was used to formally examine the relative proportions of VB17 T cells in the IL-2R+ and mIgG control samples (Table 17). Magnetic bead RNAs were reverse transcribed, preamplified with VBcon and
30 CBext and reamplified in separate reactions with a constant region primer (CBint) and each of the VB-specific primers, VB17, VB8 and VB12. The second stage amplification was also performed with two CB primers (5'CB and CBint) in order to estimate the total β -chain
35 present in each sample and to provide benchmarks for normalizing the results of specific VB quantification in

the respective IL-2R+ and control sample pairs. The proportion of VB17 DNA, relative to total CB, was increased in the IL-2R+ samples over that found in the mIgG control samples for each of the 5 patients examined.

- 5 This increase was observed in multiple analyses and the means are shown in Table 17. Enrichment was not a product of the isolation procedure, since the quantity of VB8 or VB12 DNA amplified was not significantly increased in the IL-2R+ fraction of any of the patients. Thus,
- 10 activated T cells in the rheumatoid synovium do not represent a cross section of all possible VB families, but preferentially contain VB17 T cells, and possibly other VB families which were not quantified in this analysis.

15 D. Synovial VB17 T cells display limited heterogeneity

- RNAs were reverse transcribed and amplified with the VBcons and CBext primers in 20 cycle stage I reactions and with the CBint and VBcons (1008.8) or VB8, VB12, and VB17 primers (magnetic bead RNA preparations)
- 20 in 35 cycle stage II reactions. Double stranded reaction products were electrophoresed in 2% Nu-Sieve agarose gels. After purification from gel slices with Gene Clean (Bio 101, San Diego, CA), samples were base denatured and either directly sequenced or cloned into plasmid for
- 25 sequencing of multiple independent rearrangements. In all cases, samples were sequenced from the CBseq primer (Figure 2) with T7 polymerase (Sequenase, United States Biochemicals, Cleveland, OH).

- VB17 rearrangements present in the IL-2R+ RNAs
- 30 were amplified from the VBcons and CBext preamplification with the VB17 and CBint primer pair and the reaction products sequenced. PCR products from patients 1014 and 1015 were directly sequenced and the results obtained were consistent with the presence of a single VB17
- 35 rearrangement in each of the amplified samples (Table

17). The PCR product of patient 1013 was cloned into plasmid and the thirteen isolates that were sequenced contained identical VB17 rearrangements. Sequencing of plasmid clones from patient 1012 revealed the presence of
5 two dominant rearrangements (5 isolates of each) and a single isolate of another. Thus, the VB17 repertoire in the RA synovium is of limited heterogeneity, indicative of clonal or oligoclonal expansion of VB17 T cells in vivo. This is in contrast to the VB8 and VB12
10 repertoires, from these same synovial preparations, which showed significant heterogeneity. None of the PCR-amplified VB8 and VB12 samples analyzed were directly sequenceable and plasmid cloning of VB8 rearrangements from patient 1012 revealed 4 different sequences in 5
15 clones analyzed.

VB17 rearrangements in PBLs from patient 1012 also revealed greater diversity than that seen in the synovial IL-2R+ sample. RNA from a 3 day culture of PHA/PMA stimulated 1012 PBLs was amplified with the VB17
20 primer, as for the synovial sample, and the products cloned into plasmid. Nine different rearrangements, none of which corresponded to those present in the 1012 synovium, were found in the 10 clones that were sequenced. Thus, the restricted heterogeneity of VB17
25 rearrangements in the activated synovial T cell population of patient 1012, as well as the other patients examined, likely results, not from random T cell trafficking, but from the selective expansion of those VB17-bearing T cells in the diseased tissue.

EXAMPLE XIIA. Detection of TCR β -chain transcripts in synovial T cells using V β -specific PCR-amplification

T cell receptor β -chain genes were amplified in two-stage reactions with individual V β -specific primers as described in Wucherfennig et al., *Science*, 248:1016-1019 (1990), incorporated herein by reference, and nested C β primers. RNAs were reverse transcribed (1 hour, 42°C) with the C β ext primer (40 pmol) in 12 μ l of reaction buffer (Hart et al., *supra*). Reactions were diluted with a master mix (8 μ l) containing nucleotides, reaction buffer minus MgCl₂ (final Mg⁺² concentration = 3.6 mM) and Taq polymerase, apportioned among 19 tubes containing the individual V β primers. Samples were denatured (15 minutes, 95°C) and 20 cycles of PCR were conducted. Each cycle consisted of a one minute denaturation at 95°C, a two minute annealing step and a two minute extension at 72°C. The first two cycles were annealed at 37°C and 45°C and the remainder at 50°C. One microliter aliquots of these Stage I reactions were added to 100 μ l Stage II amplification reactions (Cetus Gene-Amp Kit) containing the C β int primer (100 pmol) and 100 pmol of the V β primers used in the corresponding preamplifications. Stage II amplifications were conducted for 20 cycles with a 50°C annealing temperature and without the 37°C and 45°C ramping. Five μ l of each reaction was electrophoresed in 2% agarose gels, transferred to nitrocellulose and hybridized to a ³²P-labeled β -chain constant region probe. Blots were exposed to X-ray film and scored for the presence or absence of V β -specific amplification.

B. VB17, VB14, VB9 and VB3 transcripts are common among activated synovial T cells

To ensure that the prevalence of VB17 rearrangements in the earlier studies did not result from an amplification bias of the VB16mer primer, and to assess the presence of other VB genes in the synovium, TCR transcripts in the IL2-R+ samples were analyzed with a panel of 19 PCR primers, specific for known human VB gene families (Table 18). The VBcons primer of Example XI is equivalent to the VB16mer primer. The number of VB genes detectable in these samples was variable, ranging from two to twelve. VB17 was found in four of the five patients, confirming the previous analyses using the VB16mer primer. VB14 transcripts also were found in four of the five patients and VB3 and VB9 transcripts were detectable in three of the five samples. Thus, T cells bearing these 3 VB polypeptides may also contribute to synovial inflammation.

TABLE 18

Analysis of IL-2R+ Synovial T Cells with Individual VB-specific Primers

		VB Families																		
		1	2	3	4	5	6	7	8	9	10	11	12	14	15	16	17	18	19	20
25	<u>Patient #</u>																			
	1012			+	+													+		
	1013					+				+				+						
	1014													+				+		
	1015			+			+	+	+	+		+	+	+	+		+	+	+	
30	1020	+	+	+	+					+				+	+		+	+	+	

EXAMPLE XIIIVaccination Against EAE with a CDR4 Peptide of V88.2

Rats were immunized with 100 µg of a synthetic peptide containing the sequence VPNGYKVSRRPSQGDFFLTL (SEQ
5 ID No.46) found in the fourth hypervariable or CDR4 region of the rat TCR β chain identified as V88.2. The peptide, dissolved in saline, was emulsified in an equal volume of complete Freund's adjuvant (CFA) containing 10 mg/ul of mycobacterial tuberculosis. The animals were
10 challenged 30 days later with 50 µg of guinea pig myelin basic protein in CFA. The CDR4 peptide vaccination resulted in a reduced incidence of disease as well as a reduced severity as measured both clinically and histologically as shown in Table 19. The histology was
15 performed essentially as described in Hughes & Powell, J. Neuropath. Exp. Neurol. 43:154 (1984), which is incorporated herein by reference.

TABLE 19

Protection from EAE in the Rat by Vaccination
with a TCR-CDR4 Peptide

Vaccination	Challenge	#Clinical Signs #Tested	Mean Max Severity	Mean Onset Duration	Mean Duration	#With Histology #Tested	Mean Histology Score
Rat CDR4 in CFA	50 µg MBP/CFA	6/10	1.5	11.8	5.4	3/10	0.6
PBS/CFA	50 µg MBP/CFA	9/10	2.5	12.1	6.1	6/9	1.4
None	50 µg MBP/CFA	10/10	3.0	10.5	7.3	9/9	3.0

67

¹ rat CDR4 peptide sequence: VPNGYKVSRRPSQGDFLTL (SEQ ID No.46)

² graded on a 3-point scale as described in Example I

³ graded on a 4-point scale

Although the invention has been described with reference to the presently-preferred embodiment, it should be understood that various modifications can be made without departing from the spirit of the invention.

5 Accordingly, the invention is limited only by the following claims.

WE CLAIM:

1. A vaccine for preventing or treating a T cell mediated pathology in a vertebrate, comprising an immunogenically effective amount of at least one substantially pure T cell receptor, or a fragment thereof, corresponding to a T cell receptor present on the surface of T cells mediating said pathology, and a pharmaceutically acceptable medium.
2. The vaccine of claim 1, wherein said T cell receptor or fragment thereof comprises substantially an amino acid sequence of VB3, VB14, VB17 or any combination thereof.
3. The vaccine of claim 1, wherein said T cell receptor comprises the amino acid sequence of VB17.
4. The vaccine of claim 3, wherein said T cell mediated pathology is rheumatoid arthritis.
5. The vaccine of claim 1, wherein said fragment comprises a variable region sequence of said T cell receptor.
6. The vaccine of claim 5, wherein said variable region sequence is the β -chain variable region.
7. The vaccine of claim 6, wherein said β -chain variable region comprises substantially the amino acid sequence of VB17 or fragment thereof.
8. The vaccine of claim 7, wherein said β -chain variable region comprises substantially the sequence SQIVNDFQK.

9. The vaccine of claim 6, wherein said β -chain variable region comprises substantially the amino acid sequence of VB14 or fragment thereof.

10. The vaccine of claim 9, wherein said β -chain variable region comprises substantially the sequence SMNVEVTDK.

11. The vaccine of claim 6, wherein said β -chain variable region comprises substantially the amino acid sequence of VB3 or fragment thereof.

12. The vaccine of claim 11, wherein said β -chain variable region comprises substantially the amino acid sequence SYDVKMKEK.

13. The vaccine of claim 1, wherein said fragment comprises a V(D)J junctional sequence.

14. The vaccine of claim 1, wherein said fragment comprises a joining (J) region sequence.

15. The vaccine of claim 1, further comprising an adjuvant.

16. The vaccine of claim 1, wherein said vaccine comprises more than one type of T cell receptor or fragment thereof.

17. The vaccine of claim 16, wherein the T cell receptors or fragments thereof are selected from any combination of VB3, VB14 or VB17.

18. The vaccine of claim 1, wherein said vaccine comprises more than one fragment corresponding to different sequences of the same T cell receptor.

19. The vaccine of claim 18, wherein said fragments are selected from any combination of V β 3, V β 14 or V β 17.

20. The vaccine of claim 1, wherein said TCR fragment comprises a substantially pure peptide from a TCR β -chain region reactive with a superantigen associated with said T cell pathology.

21. The vaccine of claim 20, wherein said substantially pure peptide has an amino acid sequence of X₁-X₂-E-X₃, wherein X₁ and X₃ are each a single amino acid or a group of amino acids, and X₂ is R or K.

22. The vaccine of claim 21, wherein said amino acid sequence is substantially the sequence EGYSVSREKKESFPL.

23. The vaccine of claim 21, wherein said amino acid sequence is substantially the sequence EGYKVSRRKEKRNFL.

24. The vaccine of claim 21, wherein said amino acid sequence is substantially the sequence EGYSVSREKKERFSL.

25. The vaccine of claim 1, wherein said fragment is conjugated to a carrier.

26. The vaccine of claim 1, wherein said T cell mediated pathology is multiple sclerosis, said vertebrate is a human, and said fragment comprises substantially the sequence SGDQGGNE so as to elicit an
5 immune response against T cell receptors having substantially the sequence SGDQGGNE.

27. The vaccine of claim 1, wherein said T cell receptor comprises the sequence SGDQGGNE.

28. A method of vaccinating an individual exhibiting or at risk of exhibiting a T cell-mediated pathology, comprising administering to the individual the vaccine of claim 1.

29. The method of claim 28, wherein said vaccine is administered more than once.

30. The method of claim 28, wherein said vaccine is administered in a formulation including an adjuvant.

31. A method of treating unregulated T cell clonal replication in an individual, comprising administering the vaccine of claim 1 to the individual.

32. A method of selecting a vaccine for use in treating a T cell mediated pathology comprising the steps of:

- 5 a. obtaining T cell clones mediating said pathology;
- b. determining the amino acid sequence of T cell receptors or fragments thereof from T cell clones associated with said pathology;
- 10 c. selecting segments of those T cell receptors which are characteristic of said associated T cell receptors but not of non-associated T cell receptors; and
- 15 d. selecting amino acid sequences of said selected sequences which are capable of eliciting an immunogenic response to said T cell receptor, and thereby selecting the vaccine.

33. A method of diagnosing or predicting susceptibility to a T cell mediated pathology in an individual comprising detecting T cells having a β -chain variable region of VB3, VB14 or VB17 or a fragment thereof in a sample from the individual, the presence of abnormal expression of said VB-containing T cells indicating said pathology or susceptibility to said pathology.

34. The method of claim 33, wherein said T cells have the β -chain variable region of VB17 or a fragment thereof.

35. The method of claim 33, wherein said pathology is rheumatoid arthritis.

36. The method of claim 34, comprising detecting a portion of said VB17 which substantially does not occur on non-T cell mediated pathology associated T-cell receptors.

37. The method of claim 33, wherein said sample is from synovial tissue.

38. The method of claim 34, wherein said VB17 is detected by contacting said VB17 with a detectable ligand.

39. The method of claim 34, wherein the presence of said VB17 is detected by a nucleotide probe which is complementary to the nucleotide sequence encoding VB17.

40. A method of preventing or treating a T cell mediated pathology, comprising inhibiting the binding of a T cell receptor to its binding partner to prevent proliferation of T cells associated with said T cell mediated pathology.

41. The method of claim 40, wherein said binding inhibition is effected by a ligand reactive with the T cell receptor binding partner.

42. The method of claim 41, wherein said ligand is an antibody.

43. The method of claim 40, wherein said binding inhibition is effected by a ligand reactive with a binding site on said T cell receptor.

44. The method of claim 41, wherein said ligand is an antibody.

45. The method of preventing or treating a T cell mediated pathology of claim 40, wherein said T cell receptor is a VB3-, VB14- or VB17-containing T-cell receptor.

46. The method of claim 45, wherein said T-cell receptor contains VB17.

47. The method of claim 45 wherein said pathology is rheumatoid arthritis.

48. The method of claim 45, wherein said binding partner is an HLA-DR predisposing for rheumatoid arthritis.

49. The method of claim 46, wherein attachment is prevented by binding a ligand to VB17.

50. The method of claim 46, wherein attachment is prevented by binding a ligand to said VB17 binding partner.

51. A method of preventing or treating a T cell mediated pathology in an individual comprising cytotoxically or cytostatically treating VB3-, VB14- or VB17-containing T-cells in the individual.

52. The method of claim 51, wherein the T cells contain any combination of VB3, VB14 or VB17.

53. The method of claim 51, wherein the T cells contain VB17.

54. The method of claim 51, wherein the T cell mediated pathology is rheumatoid arthritis.

55. The method of claim 53, wherein said VB17 containing T-cells are treated with a cytotoxic or cytostatic agent which selectively binds VB17.

56. The method of claim 53, wherein said agent is an antibody attached to a moiety selected from the group consisting of radioactive moieties, chemotherapeutic moieties and chemotoxic moieties.

57. A vaccine for preventing or treating a T cell mediated pathology in a vertebrate, comprising anti-idiotypic antibodies which are internal images of a T cell receptor or a fragment thereof corresponding to a
5 cell receptor present on the surface of a T cell mediating said pathology and a pharmaceutically acceptable medium.

58. The vaccine of claim 57, wherein said fragment comprises a variable region sequence of the T cell receptor.

59. The vaccine of claim 57, wherein said fragment comprises a V (D) J junction sequence.

60. The vaccine of claim 57, further comprising an adjuvant.

61. The vaccine of claim 57, wherein said vaccine comprises anti-idiotypic antibodies which are internal images of more than one T cell receptor or fragment thereof.

62. A method of vaccinating an individual exhibiting or at risk of exhibiting a T cell-mediated pathology comprising administering to the individual the vaccine of claim 57.

63. The vaccine of claim 58, wherein said fragment comprises a junctional region sequence.

64. A method of diagnosing or predicting susceptibility to multiple sclerosis in an individual comprising detecting T cells having substantially the sequence SGDQGGNE in a sample from the individual, the presence of such T cells indicating multiple sclerosis or
5 susceptibility to multiple sclerosis.

65. The method of claim 64, wherein said sequence is detected by contacting said sequence with a detectable ligand.

66. The method of claim 64, wherein the presence of said sequence is detected by a nucleotide probe which is complementary to the nucleotide sequence encoding said sequence.

67. A method of preventing or treating multiple sclerosis comprising preventing the attachment of a T-cell receptor containing substantially the sequence SGDQGGNE to its binding partner.

68. The method of claim 67, wherein attachment is prevented by binding a ligand to said sequence.

69. The method of claim 67, wherein attachment is prevented by binding a ligand to said T cell receptor binding partner.

70. A method of preventing or treating multiple sclerosis in an individual comprising cytotoxically or cytostatically treating T cells containing substantially the sequence SGDQGGNE in the individual.

71. The method of claim 70, wherein said sequence containing T cells are treated with a cytotoxic or cytostatic agent which selectively binds said sequence.

72. The method of claim 70, wherein said agent is an antibody attached to a moiety selected from the group consisting of a radioactive moieties and a chemotherapeutic moieties and chemotactic moieties.

73. A composition of matter comprising a substantially pure T cell receptor or a fragment thereof corresponding to a T cell receptor present on the surface of T cells mediating a pathology and an adjuvant.

74. A substantially pure peptide comprising substantially the sequence SGDQGGNE.

75. A substantially pure peptide from a TCR β -chain region reactive with a superantigen associated with a T cell pathology.

76. The substantially pure peptide of claim 75, wherein said T cell pathology is rheumatoid arthritis.

77. A substantially pure peptide comprising substantially the amino acid sequence X_1 - X_2 -E- X_3 , wherein X_1 and X_3 are each a single amino acid or a group of amino acids and, X_2 is R or K.

78. The substantially pure peptide of claim 77, wherein said amino acid sequence is substantially the sequence EGYSVSREKKESFPL.

79. The substantially pure peptide of claim 77, wherein said amino acid sequence is substantially the sequence EGYKVS RKEKRNFPL.

80. The substantially pure peptide of claim 77, wherein said amino acid sequence is substantially the sequence EGYSVSREKKERFSL.

81. A vaccine for preventing or treating a T cell mediated pathology or an unregulated T cell clonal replication in a vertebrate, comprising an immunogenically effective amount of a T cell having a
5 receptor which comprises the B-chain variable region of VB3, VB14 or VB17.

82. The vaccine of claim 81, wherein said B-chain variable region is VB17.

83. The vaccine of claim 81, wherein said T cell mediated pathology is rheumatoid arthritis.

84. A method of preventing or treating an individual exhibiting or at risk of exhibiting a T-cell mediated pathology, comprising administering to the individual the vaccine of claim 81.

85. A vaccine for preventing or treating multiple sclerosis comprising an immunologically effective amount of a T cell having a receptor which comprises substantially the sequence SGDQGGNE.

86. A method of preventing or treating multiple sclerosis comprising administering to an individual the vaccine of claim 85.

87. A method of determining a T cell receptor binding partner associated with proliferation of T cells in a T cell mediated pathology, comprising:

- (a) identifying T cells that are a component
5 of the T cell mediated pathology;
- (b) determining a superantigen binding site of said T cells;
- (c) contacting a sample with said superantigen binding site; and
- 10 (d) determining the binding of said T cell receptor binding partner to said superantigen binding site, wherein binding indicates the presence of said T cell receptor binding partner in the sample.

88. The method of claim 87, further comprising administering to an individual an effective amount of said T cell receptor binding partner to prevent the proliferation of said T cells.

89. The vaccine of claim 1, wherein said immunogenic fragment comprises substantially the amino acid sequence of the CDR2 region of said β -chain variable region.

- 5 90. The vaccine of claim 89, wherein said CDR2 region comprises substantially the sequence DPGLGLRLIYFSYDVKMKEKG (SEQ ID No.75) or an immunogenic portion thereof.

- 10 91. The vaccine of claim 89, wherein said CDR2 region comprises substantially the sequence DPGLGLRQIYYSMNVEVTDKG (SEQ ID No.76) or an immunogenic portion thereof.

92. The vaccine of claim 89, wherein said CDR2 region comprises substantially the sequence DPGQGLRLIYYSQIVNKFQKG (SEQ ID No.77) or an immunogenic portion thereof.

5 93. The vaccine of claim 89, wherein said pathology is rheumatoid arthritis.

94. The vaccine of claim 93, wherein said amino acid sequence comprises substantially the sequence of VB3, VB14 or VB17.

10 95. The vaccine of claim 89, wherein said pathology is multiple sclerosis.

96. The vaccine of claim 1, wherein said β -chain VDJ region comprises substantially the sequence ASSLGGAVSYN (SEQ ID No.3).

15 97. The vaccine of claim 1, wherein said β -chain VDJ region comprises substantially the sequence ASSLGGEETQYF (SEQ ID No.4), ASSLGGFETQYF (SEQ ID No.5) or ASSLGGTEAFF (SEQ ID No.6).

20 98. The vaccine of claim 1, wherein said β -chain VDJ region comprises substantially the sequence CAIGSNTE (SEQ ID No. 2).

99. A method of preventing or treating a T cell-mediated pathology in an individual, comprising administering to the individual a nucleic acid encoding a T cell receptor or an immunogenic fragment thereof in a
5 form capable of being expressed in said individual.

100. The method of claim 99, wherein said nucleic acid is DNA.

101. The method of claim 99, wherein said nucleic acid is RNA.

102. The method of claim 99, wherein said nucleic acid is administered into a muscle of the individual.

103. The method of claim 99, wherein said immunogenic fragment comprises the amino acid sequence of a β -chain V(D)J region of said T cell receptor.

104. The method of claim 103, wherein an amino acid sequence is substantially the amino acid sequence of VB3, VB4, VB12, VB14 or VB17.

105. The method of claim 104, wherein said pathology is arthritis or multiple sclerosis.

106. The method of claim 99, wherein said immunogenic fragment comprises the amino acid sequence of a variable region of said T cell receptor.

107. The method of claim 106, wherein said variable region is a β -chain variable region.

108. The method of claim 107, wherein said amino acid sequence comprises substantially the sequence of the CDR2 region of said β -chain variable region.

109. The method of claim 107, wherein said amino acid sequence comprises substantially the sequence of the superantigen binding region of said β -chain variable region.

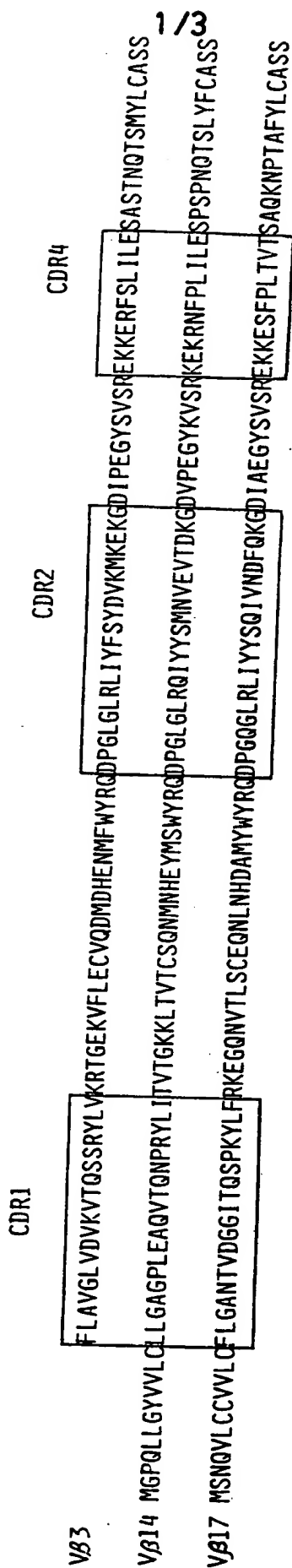
110. The method of claim 107, wherein said β -chain variable region is VB3, VB4, VB12, VB14 or VB17.

111. The method of claim 106, wherein said pathology is rheumatoid arthritis or multiple sclerosis.

112. A vector containing the nucleic acid of claim 99.

113. A composition comprising the vector of claim 99 and a pharmaceutically acceptable medium.

FIG.1



1/3

2 / 3

FIG. 2A

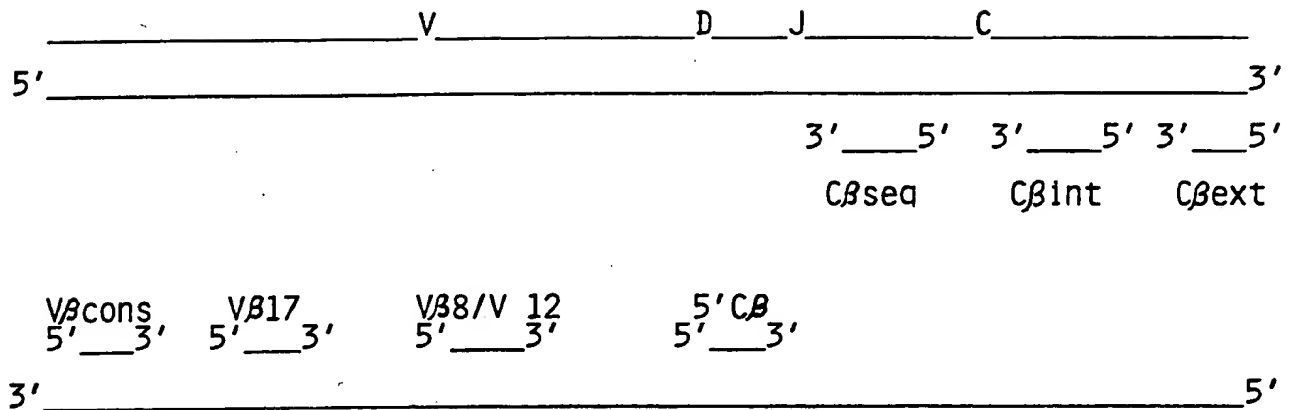


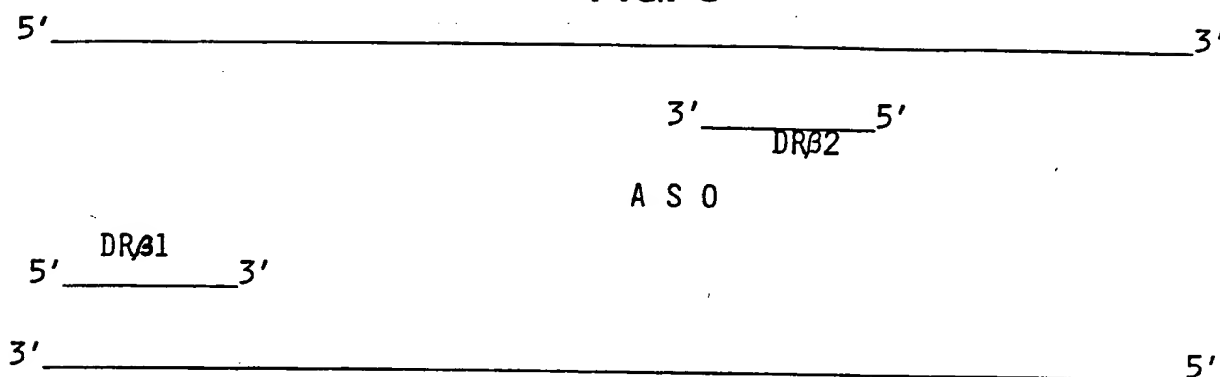
FIG. 2B

Vβcons	5' T ^{G AC} TC TGGTA ^{CAAA} CA 3'
	T TT TCGT
Vβ17	5' TCACAGATAGTAAATGACTTTCAG 3'
Vβ8	5' TCTCCACTCTGAAGATCC 3'
Vβ12	5' GATTTCCTCCTCACTCTG 3'
5'C	5' CAAGCTGTTCCCACCCGA 3'
Cβext	5' CCAGAAGGTGGCCGAGAC 3'
Cβint	5' GCGGCTGCTCAGGCAGTA 3'
Cβseq	5' CGACCTCGGGTGGGAACA 3'

SUBSTITUTE SHEET

3/3

FIG. 3



DRβ1 5' G A^A G T C T G G A A C A G C 3'
C

DRβ2 5' G T A G T^A T G T T C T G C A 3'
G

HLA-DR ALLELE-SPECIFIC OLIGONUCLEOTIDES

DRβ1 Genes

DR1, DR4w14, DR4w15	5'	CTC	CTC	GAG	CAG	AGG	CGG	GCC	GCG	3'
DR2	5'	T--	---	---	G-C	---	---	---	---	3'
DR3	5'	---	---	---	---	-A-	---	-G-	CG-	3'
DR4w4	5'	---	---	---	---	-A-	---	---	---	3'
DR4w13	5'	---	---	---	---	---	---	---	-A-	3'
DR5, DR6, DR4w10	5'	A--	---	--A	G-C	GA-	---	---	---	3'
DR7	5'	A--	---	---	G-C	---	---	-G-	CA-	3'
DR8	5'	T--	---	--A	G-C	---	---	---	CT-	3'
DR9	5'	T--	---	---	-G-	---	---	---	-A-	3'

DRβ3 Genes

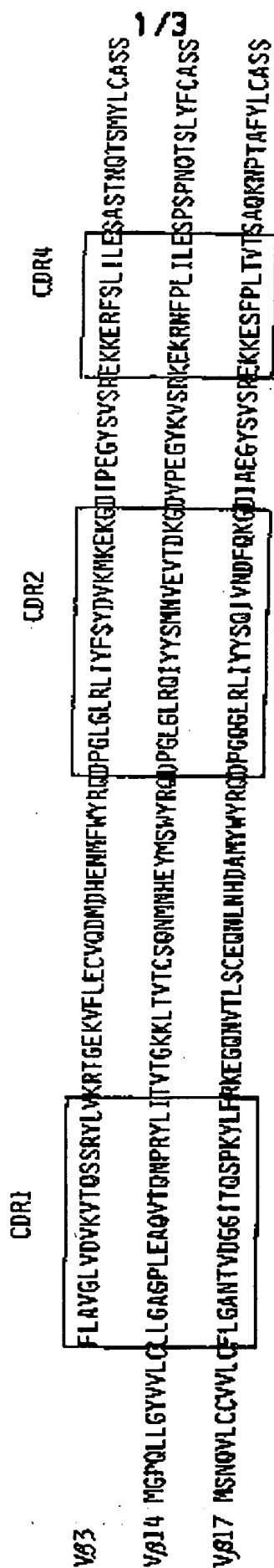
DR2	5'	A--	---	---	---	GC-	---	---	---	3'
DR3	5'	---	---	---	---	-A-	---	-G-	CAG	3'
DR7, DR9	5'	---	---	---	-G-	---	---	---	-A-	3'

PatientHLA-DR

1008	1, 4w4
1012	1, 3
1013	1, 7
1014	1, 4w4
1015	4w4, 4w4

SUBSTITUTE SHEET

FIG. 1



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FIG. 2A

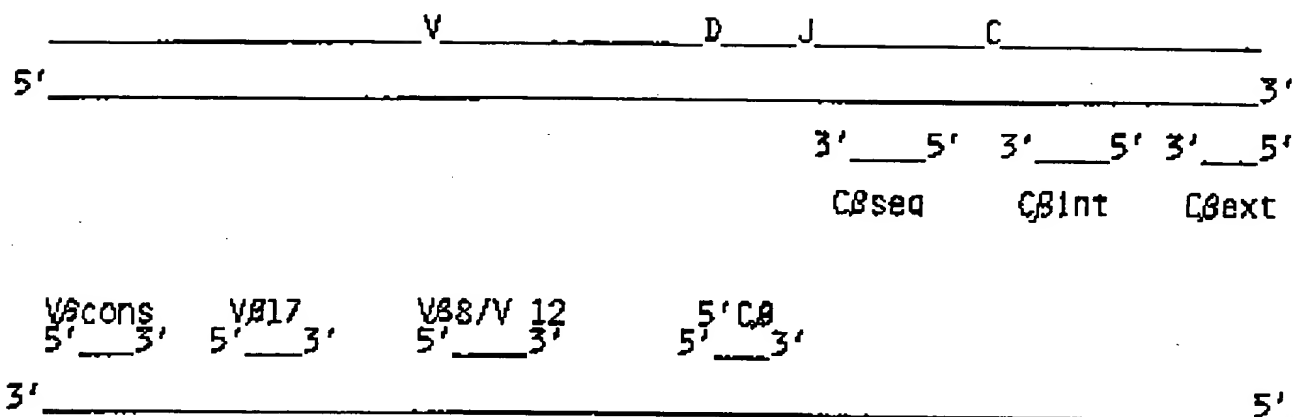


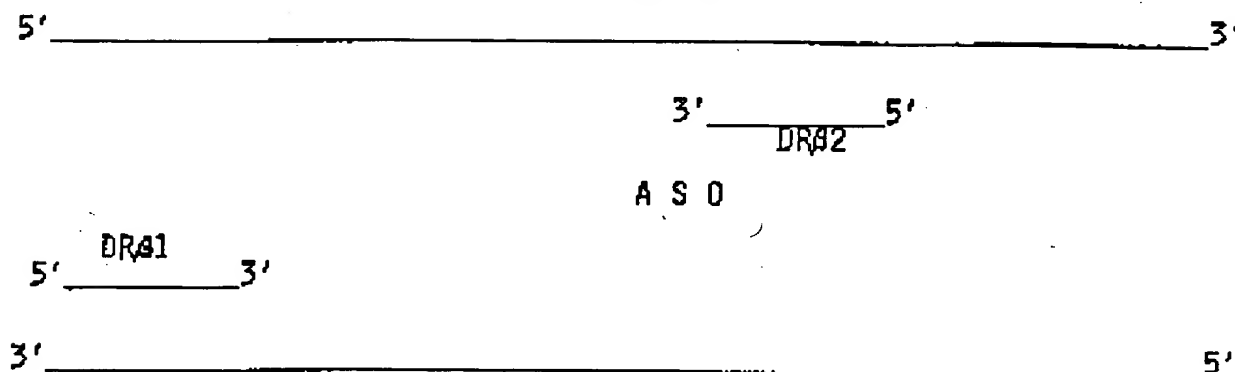
FIG. 2B

V8cons	5' T ^{G AC} TC TGGTA ^{CAAA} CA 3'
	T TT TCGT
V817	5' TCACAGATAGTAAATGACTTTCAG 3'
V88	5' TCTCCACTCTGAAGATCC 3'
V812	5' GATTTCCTCCTCACTCTG 3'
5' C	5' CAAGCTGTTCCACCCGA 3'
C8ext	5' CCAGAAGGTGGCCGAGAC 3'
C8int	5' GCGGCTGCTCAGGAGTA 3'
C8seq	5' CGACCTCGGGTGGGAACA 3'

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FIG. 3



DRB1 5' G A G T C T G G A A C A G C 3'

DRB2 5' G T A G T T G T T C T G C A 3'

HLA-DR ALLELE-SPECIFIC OLIGONUCLEOTIDES

DRB1 Genes

DR1, DR4W14, DR4W15	5'	CTC	CTC	GAG	CAG	AGG	CGG	GCC	GCG	3'
DR2	5'	T--	---	---	G-C	---	---C	---	---	3'
DR3	5'	---	---	---	---	-A-	---	-G-	CG-	3'
DR4W4	5'	---	---	---	---	-A-	---	---	---	3'
DR4W13	5'	---	---	---	---	---	---	---	-A-	3'
DR5, DR6, DR4W10	5'	A--	---	--A	G-C	GA-	---	---	---	3'
DR7	5'	A--	---	---	G-C	---	---	-G-	CA-	3'
DR8	5'	T--	---	--A	G-C	---	---	---	CT-	3'
DR9	5'	T--	---	---	-G-	---	---	---	-A-	3'

DRB3 Genes

DR2	5'	A--	---	---	---	GC-	---	---	---	3'
DR3	5'	---	---	---	---	-A-	---	-G-	CAG	3'
DR7, DR9	5'	---	---	---	-G-	---	---	---	-A-	3'

Patient	HLA-DR
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1008	1, 4W4
1012	1, 3
1013	1, 7
1014	1, 4W4
1015	4W4, 4W4

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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Published*With international search report.**Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.*(88) Date of publication of the international search report:
10 December 1992 (10.12.92)

(54) Title: VACCINATION AND METHODS AGAINST DISEASES RESULTING FROM PATHOGENIC RESPONSES BY SPECIFIC T CELL POPULATIONS

(57) Abstract

The present invention provides vaccines and a means of vaccinating a vertebrate so as to prevent or control specific T cell mediated pathologies, including autoimmune diseases and the unregulated replication of T cells. The vaccine is composed of a T cell receptor (TCR) or a fragment thereof corresponding to a TCR present on the surface of T cells mediating the pathology. The vaccine fragment can be a peptide corresponding to sequences of TCRs characteristic of the T cells mediating said pathology. Such a peptide can bind to conventional antigens complexed to MHC antigen presenting cells or to superantigens. Means of determining appropriate amino acid sequences for such vaccines are also provided. The vaccine is administered to the vertebrate in a manner that induces an immune response directed against the TCR of T cells mediating the pathology. This immune response down regulates or deletes the pathogenic T cells, thus ablating the disease pathogenesis. The invention additionally provides specific β -chain variable regions of T cell receptors, designated V β 3, V β 4, V β 12, V β 14 and V β 17, which are associated with the pathogenesis of autoimmune diseases, such as rheumatoid arthritis (RA) and multiple sclerosis (MS). Also provided are means to detect, prevent and treat RA and MS. Methods of administering DNA or RNA encoding the polypeptides useful as vaccines of the present invention into the tissue cells of an individual is also provided.

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 92/00482

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC Int.C1.5 C 07 K 7/00 A 61 K 39/00 G 01 N 33/569 C 12 Q 1/68 A 61 K 39/395		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
Int.C1.5	C 07 K A 61 K G 01 N C 12 Q	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹		
Category *	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
X	WO,A,9101133 (VANDENBARK) 7 February 1991, see claims	1-8,15- 22,25, 28,31, 32,40- 47,49- 56,73, 75-78, 81-84, 89,92- 94
X	--- WO,A,9011294 (THE IMMUNE RESPONSE CORP.) 4 October 1990, see claims ---	1-8,15- 22,25, 28,31- 58,60- 62,73,
	-/-	
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>* Special categories of cited documents: ¹⁰</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"A" document member of the same patent family</p> </div> </div>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
25-05-1992	04. 11. 92	
International Searching Authority	Signature of Authorized Officer	
EUROPEAN PATENT OFFICE	F. NOOIJ	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	<p>-----</p> <p>Nature, vol. 341, no. 6242, 12 October 1989, London (GB), A. Vandenbark et al.: "Immunization with a synthetic T-cell receptor V-region peptide protects against experimental autoimmune encephalomyelitis", pages 541-544, see the whole article</p> <p>-----</p>	<p>75-78, 81-84, 89, 92-94</p> <p>1, 2, 5, 6 , 15, 20, 25, 28, 31, 40- 45, 73, 75, 81,</p>
P, X	<p>-----</p> <p>WO, A, 9115225 (BRIGHAM AND WOMEN'S HOSPITAL; AUTOIMMUNE INC.) 17 October 1991, see claims</p> <p>-----</p>	<p>84, 89</p> <p>1-3, 5-8 , 15-19, 25, 28, 31, 33, 34, 36, 38, 39, 73, 75, 78, 81, 82, 84, 89, 92</p>

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 92/00482

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 29, 30, 102
because they relate to subject matter not required to be searched by this Authority, namely:
see PCT-Rule 39.1(iv)
Remark: Although claims 28,31,40-56,62,84,88,99-101,104-111 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition. (see also limitation due to n.u.)
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see PCT/ISA/206 mailed on 10.07.92

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

see PCT/ISA/206

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

**ANNEX TO THE INTERNATIONAL SEARCH REPORT
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US 9200482

SA 56546

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 19/10/92. The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A- 9101133	07-02-91	AU-A- 6048590	22-02-91
WO-A- 9011294	04-10-90	AU-A- 5356790	22-10-90
		EP-A- 0463101	02-01-92
WO-A- 9115225	17-10-91	AU-A- 7898991	30-10-91

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